Octadecaneuropeptide (ODN) Induces N2a Cells Differentiation through a PKA/PLC/PKC/MEK/ERK-Dependent Pathway: Incidence on Peroxisome, Mitochondria, and Lipid Profiles

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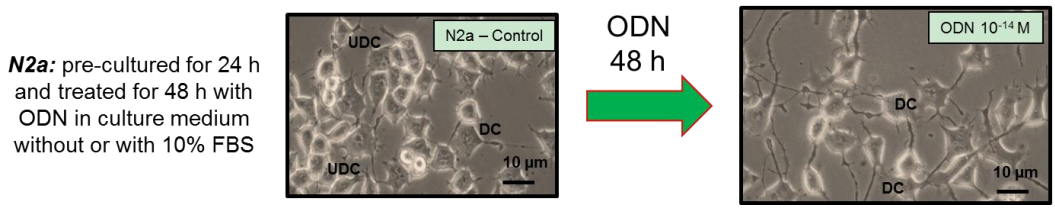
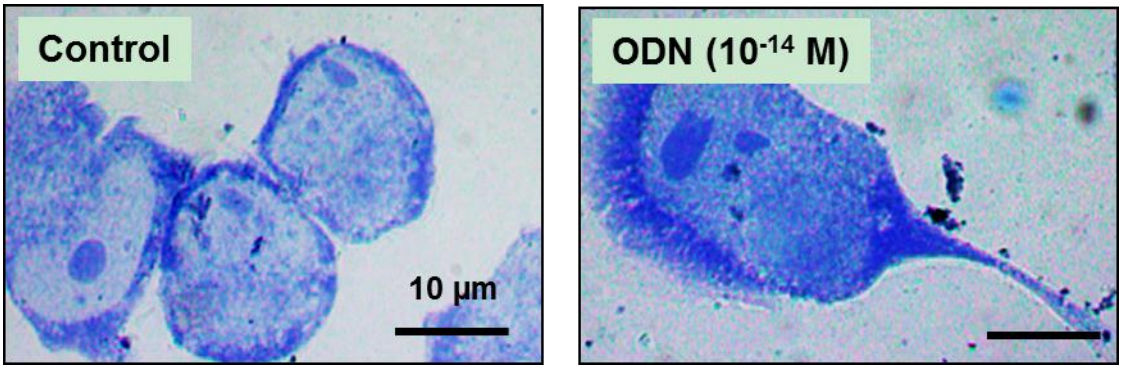
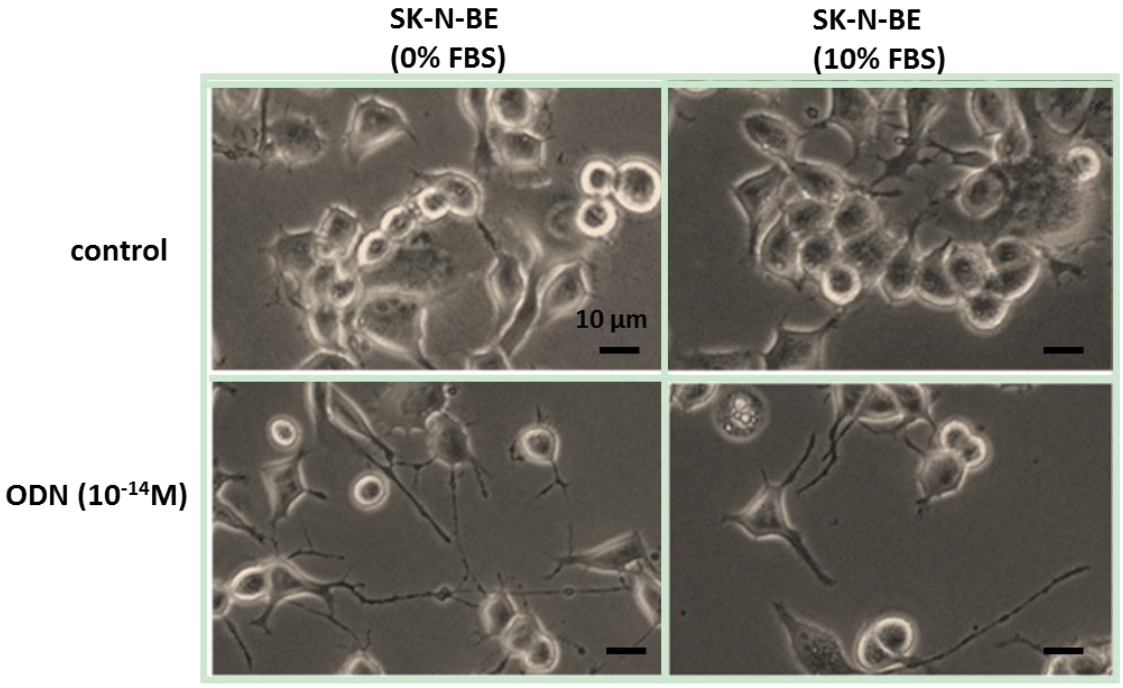


N2a cells expressing functionnal octadecaneuropeptide (ODN) receptor(s) have the ability to differentiate in neurons with neurites (dendrites and / or axons) of different length when incubated with ODN.
UDC: undifferentiated cells; DC: differentiated cells

Supplementary Figure S1: Evaluation of neuronal differentiation of N2a cells with morphological criteria by phase contrast microscopy. N2a cells (morphologically resembling neuroblasts) have the ability to differentiate in young immature and mature neurons with neurites (evocating dendrites and/or axons). Conventional morphological criteria, which were previously described [9], were used to evaluate neuronal differentiation. In control cells, mainly neuroblasts were present (cells without neurites: undifferentiated cells (UDC)). In ODN (10^{-14} M)-treated N2a cells, several differentiated cells (DC) were observed: cells with neurites of different length (average length (5-10 µm); cells with one or more neurites of important length (> 10 µm) evocating dendrites and/or axons; these neuites of important length can be associated or not with dendrites (5-10 µm length). Images were realized in phase contrast microscopy.



Supplementary Figure S2: Evaluation of neuronal differentiation of N2a cells after staining with cresyl blue. Cresyl blue is a conventional histocytological staining method which permits to detect Nissl bodies characteristic of neuronal cells. The intensity of the blue staining increases during neuronal differentiation: the cytoplasmic density of Nissl bodies is enhanced. Whereas a weak staining was observed in control cells, a strong blue staining was observed in ODN (10^{-14} M)-treated cells without FBS. The observations were realized in brightfield microscopy.



Assays	% Differentiated SK-N-BE (0% FBS)	% Differentiated SK-N-BE (10% FBS)
Control	18.73 ± 6.36	9.20 ± 5.81
ODN (10^{-14} M)	50.69 ± 3.49 **	66.19 ± 4.77 **

Supplementary Figure S3: Induction of neuronal differentiation of human SK-N-BE cells by ODN. SK-N-BE cells previously cultured in conventional medium for 24 h were further incubated for 48 h in medium without (0% FBS) or with 10% FBS in the presence or absence of ODN (10^{-14} M). Differentiated cells were characterized by neurite outgrowth. The percentage of differentiated cells was quantified from images taken under a phase contrast microscope under similar conditions than those used for N2a cells. Each value shows the mean ± SD of 5 independent experiments. Statistical analysis was performed by one-way ANOVA followed by the Bonferroni's test. ** $p < 0.01$ compared to the control.