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Endocrine Disruptors Influence Synaptogenesis in Primary Cultures of Fetal Hypothalamic Cells.

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The effects of endocrine disruptors on the mammalian central nervous system, especially in the hypothalamic area, during development remain unclear. In this study, we investigated effects of endocrine disruptors, nonylphenol and bisphenol A (BPA), on synaptogenesis in primary cultures of fetal rat hypothalamic cells. 【Methods】 Hypothalamic cells of 17 days old embryos were dispersed and placed (5 x 10^4 cells /chamber) in each chamber of culture slides coated with polyethylenimine. After incubation for three days in the normal medium, half of the medium in each chamber was changed to a medium containing nonylphenol (1, 10, 100 or 1000nM at final concentration) or BPA (1, 10 or 100μM at final concentration). Once every three days, the medium was replaced by the fresh medium containing nonylphenol or BPA. After 12 days in vitro, cells were fixed in 4% paraformaldehyde in PB, washed with PBS, subsequently incubated with primary antibodies (anti-MAP 2 monoclonal antibody and anti-Synapsin I polyclonal antibody), and stained using FITC-labeled anti-mouse IgG and Texas Red-labeled anti-rabbit IgG as second antibodies. Synapsin I-positive area and MAP 2-positive area were measured in more than 10 fields for each experimental group by a confocal laser-scanning microscope. Then, the synaptic density (Synapsin I-positive area / Map 2-positive area) was calculated. 【Results】 MAP 2-positive area was increased by 100nM nonylphenol-treatment, though other concentrations of nonylphenol did not change MAP 2-positive area. Nonylphenol also influenced Synaptin I-positive area, but in different manner. The significant increase was observed by 10nM nonylphenol, but Synapsin I-positive area was dramatically reduced by 100nM and 1μM nonylphenol. These results indicated that nonylphenol has different effects on dendritic outgrowth and on synaptogenesis. According to the change in Synapsin I-positive area, the synaptic density (Synapsin I-positive area / MAP 2-positive area) was significantly increased by 10nM nonylphenol and decreased by 100nM- and 10M nonylphenol. The significant decrease in the synaptic density was also observed after treatments with 1, 10 and 1000M BPA. Thus, these results showed that nonylphenol and BPA influence synaptogenesis in primary cultures of fetal hypothalamic cells.