Human Induced Stem Cells (hiPSCs)-Derived Motor Neurons Model Applied for The Study of the Aryl Hydrocarbon Receptor Mediated Signaling by The Induction of Environmental Pollutant

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ABSTRACT

Exposure to environmental pollutants and endogenous metabolites that induce aryl hydrocarbon receptor (AhR) expression has been suggested to affect cognitive development and, particularly in boys, also motor function. As current knowledge is based on epidemiological and animal studies, in vitro models are needed to better understand the effects of these compounds in the human nervous system at the molecular level. Here, we investigated expression of AhR pathway components and how they are regulated by AhR ligands in human motor neurons. Motor neurons generated from human induced pluripotent stem cells (hiPSCs) were characterized at the molecular level and by electrophysiology. mRNA levels of AhR target genes, CYP1A1 and CYP1B1 (cytochromes P450 1A1/1B1), and AhR signaling components were monitored in hiPSCs and in differentiated neurons following treatment with AhR ligands, 2,3,7,8,-tetrachlodibenzo-p-dioxin (TCDD), L-kynurenine (L-Kyn), and kynurenic acid (KA), by RT-qPCR. Changes in AhR cellular localization and CYP1A1 activity in neurons treated with AhR ligands were also assessed. The neurons we generated express motor neuron-specific markers and are functional. Transcript levels of CYP1B1, AhR nuclear translocators (ARNT1 and ARNT2) and the AhR repressor (AhRR) change with neuronal differentiation, being significantly higher in neurons than hiPSCs. In contrast, CYP1A1 and AhR transcript levels are slightly lower in neurons than in hiPSCs. The response to TCDD treatment differs in hiPSCs and neurons, with only the latter showing significant CYP1A1 up-regulation. In contrast, TCDD slightly up-regulates CYP1B1 mRNA in hiPSCs, but downregulates it in neurons. Comparison of the effects of different AhR ligands on AhR and some of its target genes in neurons shows that L-Kyn and KA, but not TCDD, regulate AhR expression and differently affect CYP1A1 and CYP1B1 expression. Finally, although TCDD does not significantly affect AhR transcript levels, it induces AhR protein translocation to the nucleus and increases CYP1A1 activity. This is in contrast to L-Kyn and KA, which either do not affect or reduce, respectively, CYP1A1 activity. Expression of components of the AhR signaling pathway are regulated with neuronal differentiation and are differently affected by TCDD, suggesting that pluripotent stem cells might be less sensitive to this toxin than neurons. Crucially, AhR signaling is affected differently by TCDD and other AhR ligands in human motor neurons, suggesting that they can provide a valuable tool for assessing the impact of environmental pollutants.

Keywords: AhR, CYP1A1, CYP1B1, KA, L-Kyn, hiPSCs, Motor Neurons, Stem Cell Model.