Induction of glutamine synthetase activity in primary astrocytes by Aroclor 1254 and 2,3,7,8-tetrachlorodibenzo-p-dioxin

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1. Introduction

The mechanisms underlying polychlorinated biphenyl induced developmental neurotoxicity are unclear, and may involve direct interations of PCBs with neural tissues as well as hormonal alterations[1]. The exposure of pregnant rats to the commercial PCB mixture Aroclor 1254 has been shown to alter the development of both a neurotypic and astrocytic protein (synaptophysin and glial fibrillary acidic protein, GFAP, respectively) in the brain of the offspring[2]. Either cell type may be a direct target for PCBs. Therefore we hypothesized that if PCBs have a direct effect on astrocyte development in vivo, we will be able to demonstrate that PCBs alter the development of primary astrocytes in culture at non-cytotoxic concentrations. In order to test this hypothesis we cultured primary astrocytes from the cortex and brainstem of four day old rat pups, exposed non-confluent cultures to Aroclor 1254 and TCDD, and examined two markers of astrocyte development, glutamine synthetase (GS) activity and GFAP levels.

2. Materials and Methods

Male and female Wistar WU rats (10 weeks old), were purchased from Charles River, Suzfeld, Germany. Rats were bred between 16 and 20 weeks of age, and primary astrocytes were prepared from the cortex and brainstem of four day old pups according to the McCarthy and de Vellis procedure[3] with slight modifications. The trypsinated tissue was rinsed by centrifugation, and plated at $5x10^5$ viable cells/ml in a 75 cm^2 flask. The cultures were rinsed with PBS on two consecutive days to remove debris. The culture medium, DMEM-F12 containing 10% fetal bovine serum, was changed every three days. Upon attaining confluence, the cultures were trypsinized and subcultured in 96 well plates ($5x10^5$ cells per ml) or on slides in petri dishes, allowed 24 h to attach and stretch, and were then exposed to Aroclor 1254 (1 pM to 85 μM) or TCDD (0.1 fM to 5 nM) dissolved in culture medium containing 0.25% DMSO for four to seven days. Control incubations contained the same concentration of DMSO. The purity of the cultures was examined by immunohistochemistry, using monoclonal anti-GFAP and was found to be 95% to 98% GFAP-positive cells.

Glial fibrillary acidic protein (GFAP): Cultures were washed four fold with PBS, sonicated in 200 μl PBS containing 0.1% SDS and 0.02% Tween 20. Then 100 μl of the sonicate was used

for GFAP analysis according to a method adapted from O'Callaghan[4] and with slight modifications[2] Protein concentrations were determined with the bicinchoninic acid method from Pierce[5].

Glutamine synthetase (GS) activity: GS activity was determined according to Reinhardt and Stein[6], and is expressed as the amount (umole) of glutamyl hydroxamate (GH) per well per minute. GS activity was not corrected for protein levels, however GS activity was measured after exposure to concentrations of TCDD and Aroclor 1254 which we had repeatedly determined to have no effect on protein levels.

Statistical analysis: The homogeneity of the variances and the normality of the distributions were controlled with Bartlett's test and the Shapiro-Wilks test, respectively. Significant differences from controls were determined using the Williams test (isotonic regression model).

3. Results

Brainstem or cortical astrocyte cultures exposed to DMSO or TCDD became confluent after seven days, cultures exposed to Aroclor 1254 (1pM-50 μM) reached confluence at seven days, while exposure to 85 μM Aroclor 1254 caused astrocytes to detatch and severly inhibited growth, dehydrogenase activity and proliferation (data not shown).

Table 1. Glutamine synthetase activity following exposure of cortical astrocytes to TCDD or Aroclor 1254 for seven days

log [TCDD] pM	GS activity μmole GH/well/min	log [Aroclor 1254] nM	GS activity umole GH/well/min
control	0.36 $\pm$ 0.06	control	0.51 $\pm$ 0.07
-4	0.36 $\pm$ 0.04	-3	0.69 $\pm$ 0.08
-3	0.31 $\pm$ 0.05	-2	0.79 $\pm$ 0.09*
-2	0.44 $\pm$ 0.10*	-1	0.74 $\pm$ 0.09*
-1	0.54 $\pm$ 0.04*	0	0.93 $\pm$ 0.11*
0	0.81 $\pm$ 0.08*	1	0.85 $\pm$ 0.03*
1	0.89 $\pm$ 0.05*	2	0.85 $\pm$ 0.08*
2	0.78 $\pm$ 0.06*	3	1.01 $\pm$ 0.05*
3	0.49 $\pm$ 0.05*	4	0.64 $\pm$ 0.08

Note: data are the mean $\pm$ SEM, N=6, * indicates a significant difference from controls, $P<0.05$

Exposure of cortical or brainstem astrocytes to 0.1 to 5 nM TCDD for four or seven days had no effect on total protein per well or GFAP levels (μg GFAP/mg protein, data not shown). Aroclor 1254 exposure at 50 μM for seven days increased relative GFAP levels slightly in

cortical astrocytes Exposure to 85 μM Aroclor 1254 for four or seven days was severely cytotoxic, causing drastic reductions in the number of cells and total protein per well, and reducing the relative GFAP content of both brainstem and cortical astrocytes by 90% (data not shown).

After seven days of exposure, TCDD significantly induced GS activity by 22% in cortical astrocytes at 0.1 pM, with maximal induction (140% increase) at 10 pM (Table 1). At 1 nM TCDD, GS activity had decreased relative to 10 pM, approaching control levels. TCDD had a similar biphasic effect on GS activity of cortical astrocytes at four days and brainstem astrocytes at both four and seven days (data not shown). Aroclor 1254 exposure for four and seven days also induced GS activity in both cortical and brainstem astrocytes, however, the first significant induction of GS activity by Aroclor 1254 was generally observed at 100 pM, with a peak at 1 to 10 nM. The effect of Aroclor 1254 exposure for seven days on the GS activity of cortical astrocytes is presented in Table 1.

4. Discussion

In the current study, the exposure of cortical and brainstem astrocytes to relatively high, cytotoxic concentrations of Aroclor 1254 (85 μM) severely reduced the levels of GFAP in the surviving cells. Non-cytotoxic concentrations of TCDD and Aroclor 1254 had no effect on GFAP levels of either cortical or brainstem astrocytes. In a previous study, pre- and postnatal exposure of rats to Aroclor 1254 was shown to cause long term reductions in GFAP levels in the brainstem[1]. It should be noted that the concentration of Aroclor 1254 in the culture media that reduced GFAP levels in cultured astrocytes is approximately 30,000 fold higher than the total PCBs detected in the brains of the weanling rats exposed to Aroclor 1254[1]. Therefore it seems unlikely that PCBs act directly to decrease GFAP levels in vivo, although we cannot rule out that other factors may modulate the reaction of cultured astrocytes to polychlorinated biphenyls.

The induction of glutamine synthetase activity by low concentrations of both Aroclor 1254 and TCDD indicates that both cortical and brainstem astrocytes are extremely sensitive to these compounds in vitro. The fact that Aroclor 1254 induces GS activity at 1000-fold higher concentrations as TCDD is consistent with higher general toxicity of TCDD as compared to commercial PCB mixtures. Glutamine synthetase is developmentally regulated in cultured astrocytes[7], and it is unclear if the induction of glutamine synthetase activity by Aroclor 1254 and TCDD is specific or a result of increased differentiation of the astrocytes. Although it remains to be determined if GS activity is also increased by PCB or TCDD exposure in vivo, the data indicate that astrocytes are targets for the developmental alterations caused in the CNS by PCBs.

5. References

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