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SEX-DEPENDENT CHANGES IN CEREBELLAR THYROID HORMONE-DEPENDENT GENE EXPRESSION FOLLOWING PERINATAL EXPOSURE TO THIMEROSAL IN RATS

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Mammalian brain development is regulated by the action of thyroid hormone (TH) on target genes. We have previously shown that the perinatal exposure to thimerosal (TM, metabolized to ethylmercury) exerts neurotoxic effects on the developing cerebellum and is associated with a decrease in cerebellar D2 activity, which could result in local brain T3 deficiency. We have also begun to examine TM effect on gene expression. The objective of this study was to expand on our initial observation of altered cerebellar gene expression following perinatal TM exposure and to examine additional genes that include both TH-dependent as well as other genes critical for cerebellar development in male and female neonates exposed perinatally (G10-G15 and P5 to P10) to TM. We report here for the first time that expression of suppressor-of-white-apricot-1 (SWAP-1), a gene negatively regulated by T3, was increased in TM-exposed males (61.1% increase), but not in females; ($p<0.05$). Positively regulated T3-target genes, Purkinje cell protein 2 (Pcp2; $p=0.07$) and Forkhead box protein P4 (FoxP4; $p=0.08$), showed a trend towards decreased expression in TM-exposed males. The expression of deiodinase 2 (DIO2) showed a trend towards an increase in TM-exposed females, while deiodinase 3 (DIO3), transthyretin (TTR), brain derived neurotrophic factor (BDNF) and reelin (RELN) was not significantly altered in either sex. Since regulation of gene splicing is vital to neuronal proliferation and differentiation, altered expression of SWAP-1 may exert wide ranging effects on multiple genes involved in the regulation of cerebellar development. We have previously identified activation of another TH-dependent gene, outer dense fiber of sperm tails 4, in the TM exposed male pups. Together, these results also show sex-dependent differences between the toxic impacts of TM in males and females. Interestingly, the genes that were activated by TM are negatively regulated by TH, supporting our hypothesis of local brain hypothyroidism being induced by TM and suggesting a novel mechanism of action TM in the developing brain.

Key words: *cerebellum, suppressor-of-white-apricot-1 (SWAP-1), thimerosal, thyroid hormone, brain derived neurotrophic factor, reelin*

INTRODUCTION

Thyroid hormone (TH) is critical for brain development; its deficiency during the perinatal period is associated with abnormalities in brain structure and function (1). Many factors, both genetic and environmental may contribute to TH deficiency. Of interest to this study is the contribution of TH-disrupting effect of mercury, and specifically thimerosal (TM - an ethyl mercury-containing preservative included in some vaccines administered to mothers and infants), on TH status. Surprisingly, no data on plasma TH levels following TM exposure have been reported and very few studies have explored the effect of methyl mercury (MetHg) on TH plasma levels. Studies in mice have shown that although the levels of TH in maternal and fetal plasma were not affected by short gestational exposure to MetHg, fetal brain deiodinase type 2 (D2) activity was increased (2). On the other hand, MetHg inhibited D2 activity both in neuroblastoma (3) and rat pituitary tumor cells *in vitro* (4). We have recently reported a decrease in cerebellar D2 activity following the perinatal TM exposure in SHR rats (5). Importantly, a majority of the active TH hormone in the brain is due to the activity of D2, a selenoenzyme that converts the pro-hormone thyroxine (T4) to the active hormone, 3',3,5-triiodothyronine (T3) (6); a relatively small proportion of brain T3 is transported from the plasma. Thus, it is possible that brain TH levels are altered by TM exposure, while plasma levels remain unchanged. Interestingly, T3 produced by D2 in the brain and T3 derived from the plasma are involved in the regulation of distinct gene subpopulations (7). Specifically, a deficiency of D2 results in the up-regulation of genes negatively regulated by TH (7). Thus, a decrease in D2 activity is likely to result in local hypothyroidism within the brain, and contribute to both TM and MetHg neurotoxicity through altered expression of specific subpopulation of TH-dependent genes negatively regulated by T3.

In the present study we examined this hypothesis, by assessing the effect of TM exposure on both positively and negatively regulated TH-dependent gene expression in the cerebellum. While recently (5), we reported on the effect of TM on two cerebellar genes - the Odf4 gene which was activated in males, and the cold inducible RNA binding protein (Cirbp) gene that was not affected by TM exposure (5) - present report includes data on nine additional genes that include both TH-dependent genes as well as other genes critical for cerebellar development. We report here for the first time up-regulation of a gene negatively regulated by T3