Consequences Of Rat Perinatal Exposure To Mixtures Of Polychlorinated Biphenyls Or Organochlorine Pesticides On Thyroid Hormone System And Hippocampus Protein Profile

Guillaume Pelletier[1], Sheila Masson[1], Mike Wade[1], Wayne J Bowers[1], Nanqin Li[1], Ih Chu[1]

[1]Health Canada

Introduction

Epidemiological studies have correlated high fish consumption by mothers to perturbation of neurological functions in their infants[1] and laboratory studies have linked rat exposure to single polychlorinated biphenyl congeners or technical mixtures to learning/memory and motor activity deficits[2]. Since humans are exposed to mixtures of chemical pollutants rather than single chemicals, it is important to conduct toxicity studies on mixtures. This project was therefore designed to investigate the toxicity resulting from perinatal exposure to low levels of environmentally relevant mixtures of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) that approximate the exposure profile of Canadian Arctic populations. In addition, given that PCBs interfere with thyroid functions[3], a positive control group for hypothyroxinemia was added by exposing dams to 6-propyl-2-thiouracil (PTU). Pup growth, mortality, serum T4 and TSH levels, thyroid gland morphology and brain protein pattern resulting from exposure to PCBs, OCs and PTU were compared.

Materials and Methods

Details of rat handling, identification and breeding were described in Bowers et al.[4] Sprague-Dawley rat dams were exposed to a mixture of 14 polychlorinated biphenyl congeners or to a mixture of 12 organochlorine pesticides that represents the most abundant organochlorine pollutants present in the blood of Canadian Arctic populations[5]. PCBs (congener 153, 138, 180, 99, 187, 118, 170, 156, 183, 105, 52, 101, 128 and 28) and OCs (hexachlorobenzene, trans-nonachlor, p,p'-DDE, oxychlordane, b-HCH, toxaphene, p,p'-DDT, cis-nonachlor, mirex, heptachlor epoxide and aldrin) were dissolved in corn oil, and a weight adjusted volume was dispensed onto a Teddy Graham cookie (Nabisco) provided to the dams daily from gestation day 1 to post-natal day (PND) 21. Dams were dosed with 0.011 and 1.1 mg PCBs/kg body weight and with 0.019 and 1.9 mg OCs/kg body weight. In a parallel study, dam serum contaminant concentrations following similar exposure to PCBs and OCs closely matched the serum profile of Canadian Arctic mothers (unpublished data). The hypothyroxinemia control group was dosed with 0.001% PTU in drinking water from gestation day 6 to PND 21. Pups were counted at birth and mortality recorded and culled to 4 males and 4 females per litter on PND 4. Necropsies were performed on PND 14 and PND 21. Pups designated for brain analysis were decapitated without anaesthesia and the brain quickly extracted, rinsed with ice-cold PBS, cut in half along the longitudinal fissure, frozen on crushed dry ice and kept at -80 ^{o}C. Brain substructures were dissected afterward on thawing half-brains. For thyroid analysis, pups were anaesthetized prior to thyroid gland and blood collection. Serum T4 and TSH concentrations were measured according to Bowers et al.[4] Hippocampus or cerebellum protein extracts were passively absorbed on Readystrip IPG strip pH 5-8 (Bio-Rad), electrofocussed on Protean IEF cell (Bio-Rad), electrophoresed on Miniprotean III (Bio-Rad) and stained with Bio-Safe Coomassie (Bio-Rad) according to the manufacturer's protocols. Gels were digitalized with a GS-800 calibrated densitometer (Bio-Rad). Phoretix software (Nonlinear Dynamics) was used to quantify and analyse data. Due to the inherent variability of 2D gels, at least 10 samples per treatment group were analysed. Spots were considered as significantly affected only if they consistently showed variation of intensity greater than two-fold using any combination of 3 methods of background subtraction and 2 methods of normalization.

Results and discussion

Litter size and sex ratio were not affected in any treatment group (data not shown). Pups mortality in the high dose OCs treatment group before culling was similar to the vehicle control group with mortality rates of 2.2% and 3.1% respectively and no mortality was recorded afterward. High dose PCBs and PTU treatment groups induced similar mortality rates of 5.5% and 8.7% before culling and 1.3% and 2.3% after culling, respectively.

Exposure to OCs failed to affect serum T4 while the effect of high dose PCBs exposure on serum T4 concentration was roughly similar to the effect of PTU at PND 14 and 21 (Figure 1A, 1B). As previously reported[6,7], PCB-induced decrease in serum T4 failed to increase serum thyroid stimulating hormone (TSH) to the same extent as PTU-induced hypothyroxinemia (Figure 1C). Another noticeable difference between the PTU and high dose PCBs treatment groups was the failure of the latter to affect pups weight gain (Figure 1D).

Analysis of male PND 14 hippocampus protein patterns revealed striking similarities between PBCs and OCs high dose treatment groups: out of a total of 7 differentially expressed proteins, 4 spots (A, B, C and F) were differentially expressed in both PCBs and OCs treatment groups (Table 1). The approximately 400 proteins resolved on 2D gels represent only a small fraction of the proteins expressed in the brain, but it can be assumed that a significant fraction of the effect of PCBs on hippocampus proteome is not thyroid hormone dependant (as OCs failed to affect the thyroid hormone system). This finding corroborates other reports on the

differential effects of PCB and hypothyroxinemia on the timing of eye opening developmental landmark[8] and on neurotransmitter levels[7].

The differentially expressed proteins shared by the PCBs and OCs high dose treatment groups are unlikely to be good biomarkers of neurotoxicological effects as perinatal exposure to the OCs mixture failed to affect neurobehavioural parameters (W.J. Bowers, personal communication). Alternatively, the 4 differentially expressed protein spots shared by PCBs and OCs might constitute potentially interesting biomarkers of exposure to a wide variety of chlorinated organic molecules. However, their use in more complex contaminant mixtures might be limited as perinatal co-exposure of PCBs and OCs mixtures with 2 mg methyl mercury/kg body weight resulted in the differential expression of spots A and G only (unpublished data).

In order to further untangle the effects of the PCBs mixture on thyroid hormone from the other neurotoxic effects, we will complete the proteomic analysis of the cerebellum from the PCBs and PTU treated groups and identify differentially expressed proteins by MALDI-ToF protease fingerprinting. Microarray hybridisation of cerebellum and hippocampus mRNA is also underway. Correlation of this wide array of molecular data with neurobehavioural endpoints measured on similarly treated rats will allow us to better understand the role and relative importance of hypothyroxinemia in PCB-induced neurotoxicity.

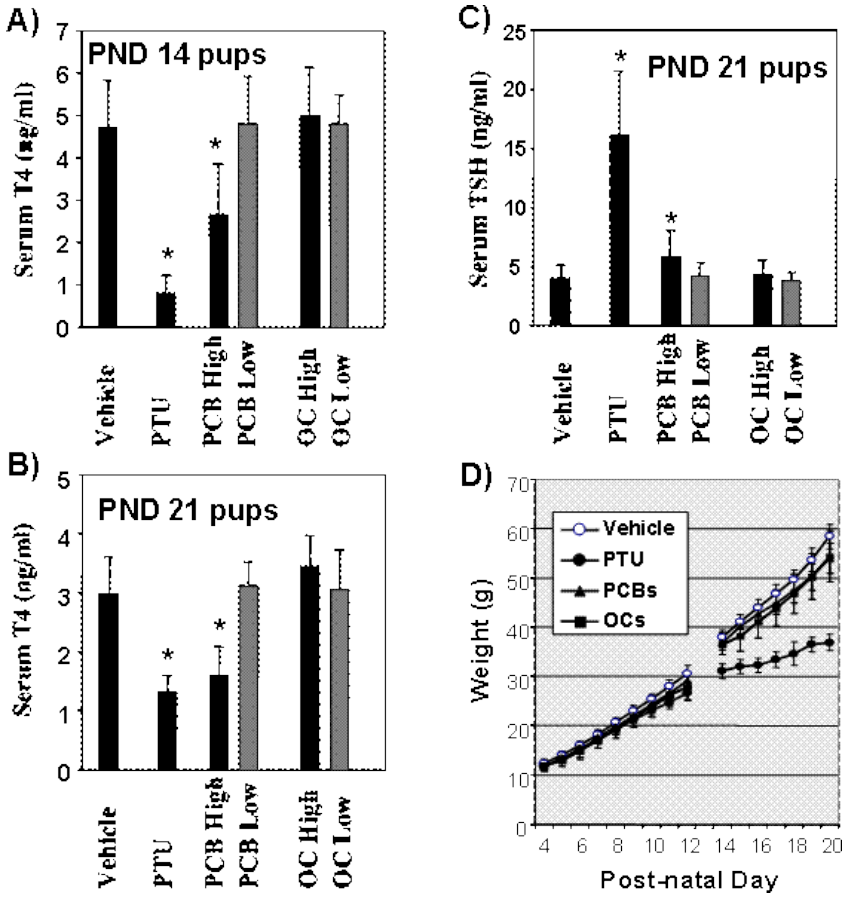

Figure 1: Serum T4 concentration in A) PND 14 and B) PND 21 pups. C) Serum TSH concentration in PND 21 pups. D) Pup weight gain from PND 4 to PND 21 (only the high dose treatment groups are shown). Error bars are standard deviation and * indicates statistically significant difference from the control group ($p < 0.05$).

			Rel. Expression	
Spot	m.w.	I.P.	PCBs	OCs
A	70	6.0	**2.82**	**5.24**
B	38	6.85	**2.82**	**2.90**
C	38	7.0	**2.37**	**3.66**
D	35	5.55	0.80	**0.42**
E	32	6.85	**2.57**	1.98
F	27	6.75	**4.60**	**4.65**
G	24	6.4	**0.33**	0.70

Table 1: List of differentially expressed protein spots following exposure to high dose PCBs and OCs mixtures. The apparent molecular weight (m.w.) and isoelectric point (I.P.) for each spot are listed. The expression values in the vehicle control group were arbitrarily set to one and variations of relative expression greater than two-fold are indicated in bold prints.

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