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NEUROCHEMICAL EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO A MIXTURE OF PERSISTENT ORGANIC POLLUTANTS AND MERCURY BASED ON BLOOD PROFILES OF ARCTIC POPULATIONS

J.S. Nakai[1], W.J. Bowers[1], I. Chu[1], M. Wade[1], D. Moir[1], O. Pulido[2], S. Gill[2] & R. Mueller[2]

[1] Environmental Health Sciences Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Environmental Health Centre, Tunney's Pasture, Ottawa, ON K1A 0L2, Canada
[2] Toxicology Research Division, Health Products and Foods Branch, Health Canada, Banting Research Centre, Tunney's Pasture, Ottawa, ON K1A 0L2, Canada

Introduction

The health of people living in Arctic regions may be adversely affected because their traditional diets have high concentrations of environmental toxicants [1]. Fetuses and infants may be especially vulnerable to the effects of exposure since their tissues and organs are undergoing periods of rapid growth and development. For example, epidemiological evidence suggests that the children of mothers exposed to persistent organic pollutants (POPs) or toxic metals such as mercury may be at risk of suffering from intellectual impairments, behavioural changes or reduced growth [2,3]. Similarly, maternal exposure of laboratory animals to polychlorinated biphenyls (PCBs) or methyl mercury can affect emotional and motor functions, activity levels and behaviour in their offspring [2-4]. Developmental exposure to environmental pollutants such as PCBs also affects a wide range of biochemical measurements. In terms of neurochemistry, experimental evidence suggests that: 1) the effects of PCBs on adult animals differ from those on offspring; 2) mixtures of PCBs may act additively or synergistically; and 3) PCBs and methylmercury can act synergistically to affect dopamine levels [2,5,6]. Neurotransmitters such as dopamine have important roles in motivated behaviours, motor behaviour, behavioural sensitization, schizophrenia and cognition and in disorders such as Parkinson's disease [7-9]. The goal of this work was to examine the neurochemical effects of gestational and lactational exposure of rat pups to a mixture of POPs and mercury based on blood levels of Arctic populations.

Methods and Materials

Sprague-Dawley rats (females weighing 200-230 g, males weighing 315-350 g) were received at the Animal Resources Division of Health Canada from Charles River Laboratories (St. Constant, Quebec) and allowed to acclimatize for at least three weeks. The animals were bred and the "pregnant" rats randomly assigned to the vehicle control group (corn oil), positive control group (15 mg/kg/day Aroclor 1254) or one of three dose groups of the Arctic mixture (0.05, 0.5 or 5 mg/kg/day). This mixture was composed of 14 PCB congeners (21% by weight), 12 different organochlorines (41%) and methylmercury chloride (38%). The proportion of each component was based upon the geometric mean blood levels of Arctic populations, taken from the Northern Contaminants Program database, except for methylmercury, which was based on the median blood levels of Inuit populations. The dams were dosed from gestation day 1 through weaning (postnatal day 23); the pups were never dosed directly. A measured aliquot of the Arctic mixture or Aroclor

1254, based on the daily body weight of each animal, was administered by applying the dose to a "Teddy Graham" cracker (Nabisco Ltd., Toronto) which was then fed to the rats. Litters were culled to four males and four females on postnatal day 4 (PND 4) and were evaluated using a variety of neurobehavioural and developmental tests. One male and one female from each litter were sacrificed for tissue analysis as a juvenile (PND 35 – results presented here), as a young adult (PND 75) or as a mature adult (PND 350), and in addition to the neurochemical analysis, systemic, endocrine, molecular and histopathological endpoints were also examined; these results will be reported separately. The animals were sacrificed by decapitation, the brains were immediately removed and a mid-sagittal section was made. The brain halves were frozen on powdered dry ice and stored at -80°C until analysis. Either the left or right side was subjected to neurochemical analysis, counterbalanced between dose groups and sex. The neurochemical method employed was essentially that of Seegal *et al.* [10]. Briefly, the frozen brains were cut into 750 μm slices and tissue punches (14-16 gauge) were obtained from the frontal cortex, caudate nucleus, nucleus accumbens, hippocampus and substantia nigra. These regions were selected because they are known to be affected by PCB treatment or mercury treatment [11-14]. The concentrations of norepinephrine (NE), 3,4-dihydroxyphenylalanine (DOPA), dopamine (DA), 3-methoxytyramine (3-MT), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in each tissue punch were determined by HPLC with electrochemical detection and the protein content measured using a modified Lowry assay (Sigma-Aldrich Chemical Ltd., Oakville). The results are reported as ng neurotransmitter per mg protein. Data were examined using one-way analysis of variance (ANOVA) with post-hoc analysis by Dunnett's two-sided test. Gender differences and lateralization effects were investigated using T-tests. Significant changes were considered at the $p<0.05$ level.

Results and Discussion

Gestational and lactational exposure to either the Arctic mixture or Aroclor 1254 exerted a significant effect on neurotransmitter levels of 35 day old Sprague-Dawley rat pups (Figure 1) but the effect of the mixture was distinct from that of Aroclor. The Arctic mixture caused a dose-dependent decrease in 5-HIAA that was observed in the caudate nucleus for both males and female PND 35 pups (Figure 1). However, 5-HT in the caudate nucleus and HVA and DA in the nucleus accumbens were significantly affected only for the female pups. In all cases, similar trends were observed for the males but did not reach statistical significance. Gender differences and lateralization effects from exposure to the Arctic mixture were minimal with small but statistically significant changes observed only in the nucleus accumbens and substantia nigra (Figure 2).

While Aroclor 1254 had an effect only on the nucleus accumbens of the PND 35 pups, gestational and lactational exposure to the Arctic mixture caused significant changes in neurotransmitter levels in both the caudate nucleus and nucleus accumbens. This was observed despite the fact that the Aroclor was administered at 15 mg/kg/day while the Arctic mixture was administered at 0.05, 0.5 or 5 mg/kg/day; effects were observed even at the lowest dose level which was 300 times less than that for Aroclor and 1,400 times less if only the PCB content of the mixture was considered. All three fractions of the mixture (PCB, organochlorine, methylmercury) are known to affect these neurotransmitters and it is possible that the various mixture components work additively or synergistically to affect the neurotransmitter levels in this work [2,15,16]. Previously, Bemis and

Seegal have found that the combination of PCBs and methylmercury had a synergistic effect in lowering rat brain DA content *in vitro* [6].

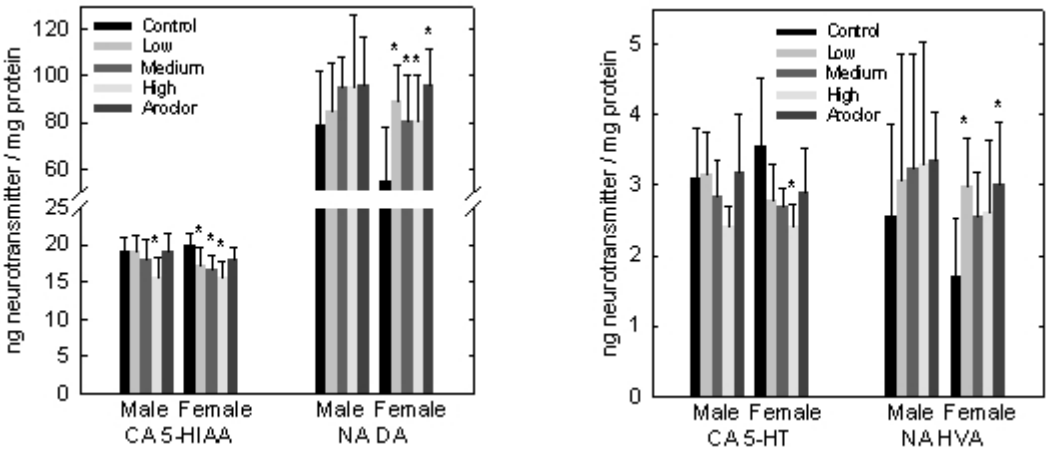

Figure 1: Significant Changes in Neurotransmitter Levels. The two panels show the significant changes in neurotransmitter levels in the caudate nucleus (CA) and the nucleus accumbens (NA). The data are average ± standard deviation and * indicates the dose groups which were significantly different from the controls at $p<0.05$.

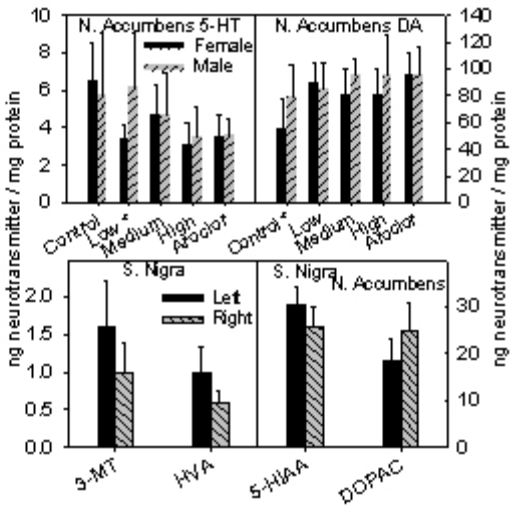

Figure 2: Gender and Lateralization Effects: Significant differences (*) between genders were observed in the nucleus accumbens for 5-HT in the low dose animals (upper left) and for DA in the controls (upper right). A significant difference between the left and right side of the brain was detected for a number of neurotransmitters in the substantia nigra: 3-MT in the control animals, HVA in the medium dose animals (bottom left) and 5-HIAA in the controls (bottom right). A significant lateralization effect was also observed for DOPAC in the nucleus accumbens of the medium dose animals (bottom right). The data are average ± standard deviation and significant differences were considered at $p<0.05$.

The Arctic mixture was observed to significantly affect neurotransmitters of PND 35 rat pups in both the caudate nucleus and nucleus accumbens at levels as low as 0.05 mg/kg/day. Comparison with the pups sacrificed at PND 75 and PND 350 will determine whether the effects observed are persistent, or whether additional effects emerge with time. Finally, these data will be integrated with reproductive, developmental, behavioural, endocrine, systemic, molecular and histopathological data from these same animals to provide public health officials with a better understanding of the potential health effects of exposure of Arctic populations to these pollutants.

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