POLYCHLORINATED BIPHENYLS AND METHYLMERCURY INCREASE SYNAPTOSOMAL MEDIA DOPAMINE CONCENTRATIONS BY DIFFERENT MECHANISMS

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Introduction

Epidemiological studies have demonstrated an association between consumption of contaminated fish by women of child-bearing age and behavioral deficits in their infants and children.[1-3] Because polychlorinated biphenyl (PCB) body burdens are statistically associated with these deficits it has often been assumed that PCBs are responsible for the observed deficits. Contaminated fish and marine mammals, however, contain a large number of other potentially neuroactive contaminants, including methylmercury (MeHg),[4] that may not only contribute to the observed deficits but may also interact to exacerbate the deficits suggested to be due to exposure to PCBs.[5]

We have recently demonstrated, using striatal tissue derived from adult rat brain, that PCBs and MeHg act synergistically to alter synaptosomal dopamine (DA) function, including reductions in synaptosomal DA content and elevations in media DA. However, there have been to the best of our knowledge no studies that have examined the neurochemical consequences of exposure to these contaminants in tissue from early post-weaning rats. The studies, described below, compare the effects of exposure of striatal synaptosomes from postnatal day (PND) 7, 14, 21 and 70 (adult) rats on media DA content following *ex vivo* exposure to either PCBs or MeHg. We have chosen to focus on this measure because changes in the handling and storage of DA have profound influences on neurochemical and behavioral function.[6] Information gathered from these experiments will aid in understanding the risks associated with developmental exposure to these structurally-disparate contaminants and may suggest modifications to existing fish consumption guidelines.

Materials and Methods

The PCBs used in these experiments consisted of a mixture of Aroclors designed to mimic the exposures seen in anglers who consume fish from the Fox River (FR) area of Wisconsin.[7] Briefly, this mixture (FR mix) consisted of 35% Aroclor 1242, 35% Aroclor 1248, 15% Aroclor 1254 and 15% Aroclor 1260. FR PCBs were dissolved in dimethylformamide (DMF) to create 1000x concentrated stock solutions that were then used to prepare media containing PCBs at the appropriate concentrations (10, 20 or 40 μM). MeHg was also dissolved in DMF and diluted in growth medium to achieve final media concentrations of 1.0, 2.5, 5 or 10 μM. In all instances, concentrations of DMF in growth medium were no more than 0.2%.

Striatal synaptosomes were prepared according to methods described by Loscher *et al.*[8] and were exposed to either PCBs or MeHg for 30 minutes. Following exposure, concentrations of DA in media from both control and exposed synaptosomes were analyzed by HPLC-EC according to previously described methods.[9] In addition, we determined MeHg-induced increases in reactive oxygen species (ROS) formation in synaptosomes from both early post-weaning and adult rats by measuring 2',7'-dichlorofluorescein (DCF) concentrations using procedures described by Myhre *et al.*[10] Elevations in DCF concentrations occur in the presence of hydroxyl radicals, perioxynitrite or hydrogen peroxide.

Results

Media DA Concentrations

FR PCBs significantly increased media DA concentrations in synaptosomes obtained from rats of all ages (Figure 1A)—a not unexpected finding in light of recent findings from Mariussen and Fonnum[11] and Bemis Seegal[9] demonstrating that PCBs inhibit DAT function, including uptake of labeled DA. In contrast, MeHg significantly elevated media DA concentrations only in synaptosomes derived from PND7 rats (Figure 1B), suggesting that DAT inhibition is not a primary mechanism by which MeHg alters DA function.

Figure 1A

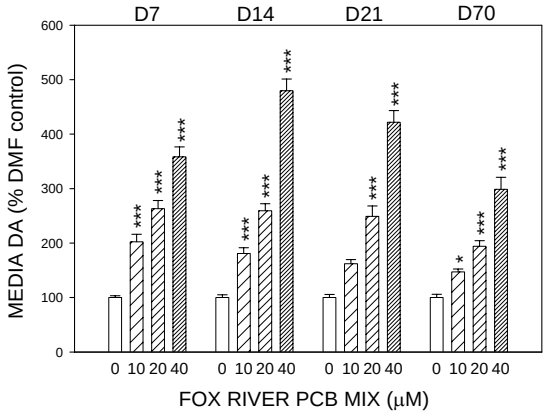

Figure 1B

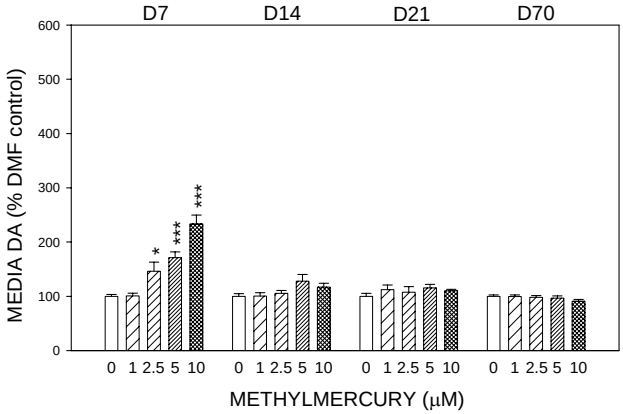

Figure 1. PCBs induce dose-dependent increases in media dopamine at all ages, while methylmercury is active only at the youngest age. Dose-response relationships for dopamine release (media DA) using micromolar concentrations of Fox River PCB mixture **(A)** and methylmercurcy **(B)** in P3 fraction synaptosomes prepared from the striatum of postnatal day 7, 14 or 21, or adult (70 days of age) rats. *$p \leq 0.05$, ***$p \leq 0.001$; indicates a significant post-hoc *t*-test comparison for either the Fox River PCB mixture or methylmercury treatment with respect to the age-matched DMF vehicle control (N= 9-30 samples per treatment group).

Methylmercury Increases Oxidative Stress Primarily in Synaptosomes from Early Post-Weaning Rats

MeHg exposure increased ROS formation in synaptosomes from rats of all ages with the highest levels observed in synaptosomes from the younger rats (Figure 2). The MeHg-induced elevations in ROS were strictly age-dependent with MeHg concentrations as low as 1μM significantly increasing ROS levels in PND7 synaptosomes.

Figure 2

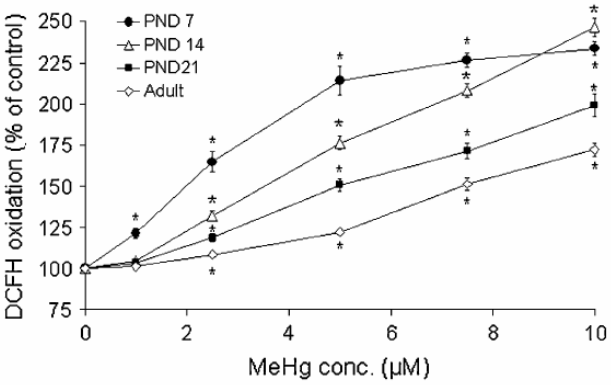

Figure 2. Methylmercury (MeHg) induces greater ROS formation in striatal synaptosomes from younger rats. Striatal synaptosomes were loaded with DCFH-DA and exposed to MeHg for 30 min before ROS formation was assessed by measuring increases in DCF fluorescence. Data is presented as a percentage of age-matched vehicle controls. *$p \leq 0.05$; significantly different from age-matched controls using Dunnett's post hoc *t*-test (N=16-22 wells per treatment group from 3-4 experiments).

Discussion

The most unexpected finding—that MeHg elevated media DA concentrations only in PND7 synaptosomes—stands in sharp contrast to the dose, but not age-dependent, increases in media DA seen in synaptosomes exposed to FR PCBs. These differences in effects on media DA—an endpoint most often associated with inhibition of the DAT[12]—suggest that these two contaminants affect DAT function by different mechanisms. As mentioned previously, PCBs have been shown to inhibit monoamine transporters, including the DAT, in a number of *in vitro* preparations.[9,11] Thus, the elevations in media DA concentrations following exposure to FR PCBs are likely to be the result of direct inhibition of the DAT.

The reasons for MeHg-induced elevations in media DA, *seen only in PND7 synaptosomes,* are less well understood. We hypothesize that the MeHg-induced increases in media DA are not due to a direct inhibition of the DAT because, if they were, elevations in media DA concentrations should be evident in synaptosomes derived from rats of all ages. Instead, because MeHg induces mitochondrial ROS formation to a greater extent than PCBs,[13] we first suggest that developmental differences in activities and/or levels of enzymes that detoxify ROS are responsible for the greater increases in ROS seen in synaptosomes from younger rats compared to increases seen in adult rats. In turn, elevations in oxidative stress inhibit uptake of labeled DA by the DAT,[14] providing a link between the higher levels of ROS and elevations in media DA concentrations seen in PND7 synaptosomes. The lack of an increase in media DA in PND14 synaptosomes, despite significant MeHg-induced elevations in ROS, may, at first glance, be puzzling. Nevertheless, we suggest that the almost two-fold increase in striatal DAT densities from PND7 to PND14, levels that approximate those seen in the adult striatum, (Galineau *et al.*[15]) mitigate the effects of ROS on DAT function. Thus, in addition to lower levels of anti-oxidant defense mechanisms, lower densities of monoamine transporters may contribute to the greater sensitivity of the immature central nervous system to environmental neurotoxicants.

In summary, despite evidence that both PCBs and MeHg increase media DA concentrations, we suggest that they do so by totally different mechanisms—PCBs by direct competitive inhibition of DAT and MeHg by indirectly elevating ROS, which in turn, results in DAT inhibition in immature brain tissue. Thus, knowledge of developmental changes in nervous system function is critical to understanding the mechanisms and risks associated with exposure to these and other environmental contaminants.

Acknowledgement

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