Polychlorinated Biphenyls Interact with Other Great Lakes Fish-Borne Contaminants to Alter Dopamine Function *In Vitro*

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Introduction

Epidemiological studies have demonstrated an association between consumption of contaminated fish by mothers and deficits in growth, development and cognition in their infants and children (1,2). PCB concentrations in either breast milk or fetal cord blood have been associated with these deficits suggesting that PCBs may be the contaminant responsible for these deficits. However, contaminated Great Lakes (GL) fish, including salmon, contain many other known and suspected developmental neurotoxicants, including methylmercury (MeHg), lead and chlorinated pesticides (3). In turn, it is highly likely that the concentrations of these contaminants co-vary with PCB levels found in the fish making it difficult to determine which contaminant(s) is responsible for the observed association between maternal fish consumption and deficits in their infants and children.

In order to begin to determine which contaminants alter central nervous system (CNS) function we have examined changes in tissue and media concentrations of dopamine (DA) following *in-vitro* exposure of striatal punches from adult rats to PCBs alone, or in combination, with some of the major neurotoxicants present in contaminated GL salmon. We have chosen to: (i) employ striatal punches rather than cells in culture since the explanted tissue conserves much of the neuronal complexity and neurotransmitter interactions that are present in the intact brain and (ii) examine changes in DA function since one of the major actions of PCBs, both in culture and the intact preparation, is to alter the activity of this neurotransmitter.

Experimental Methods

Male Wistar-derived rats, approximately 90 days of age, were decapitated and their brains removed and placed in ice-cold saline. Each forebrain was mounted on a vibratome and 350 µm thick slices were taken through the striatum. The slices were transferred to a petri dish containing ice-cold Hepes-buffered Hank's saline (HBHS) and two 4-mm diameter punches were obtained from each slice. The resulting punches were individually placed in 24 well culture plates containing 500 µl of HBHS supplemented with 1% horse serum containing 0.2% dimethylsulfoxide (DMSO) or 0.2% DMSO containing the compounds of interest. The

punches were incubated for four hours in a 37°C shaking water bath under an atmosphere consisting of 95% O_2/5% CO_2. Following incubation, media, diluted 1:1 with 0.4 N perchloric acid and punches, homogenized in 0.2 N perchloric acid, were collected for analysis of DA by high performance liquid chromatography with electrochemical detection (4).

The contaminants tested included PCBs (a 1:1 mixture of Aroclors 1254 and 1260 at concentrations of 1, 10 or 100 ppm), mirex, DDE, chlordane or MeHg. The latter test compounds were presented at concentrations of 1 or 10 μM.

Results and Discussion

Exposure of striatal punches to the Aroclor mixture resulted in significant, dose-dependent decreases in DA concentrations (F=19.34, df=3,32, $p\leq0.001$) and significant, dose-dependent increases in media DA concentrations (F=15.73, df=3,32, $p\leq0.001$) — results similar to those reported by Chishti *et al.* (5), (data not shown).

MeHg, mirex, DDE and chlordane, when presented alone, at the above concentrations, failed to alter either punch or media concentrations of DA. However, co-exposure of striatal punches to 100 ppm of the Aroclor 1254/1260 mixture and MeHg significantly decreased punch DA concentrations (F=17.7, df=2,78, $p\leq0.001$) and significantly elevated media DA concentrations (F=4.55, df=2,78, $p\leq0.01$), compared with exposure to only the PCBs at the same concentration, (Fig. 1). Similarly, chlordane, when combined with the Aroclor 1254/1260 mixture, significantly reduced punch DA concentrations (F=10.29, df=2,108, $p\leq0.001$) and significantly elevated media DA concentrations (F=11.82, df=2,105, $p\leq0.001$) compared with exposure to only the PCB mixture, (Fig. 2). Neither DDE nor mirex, when combined with the Aroclor 1254/1260 mixture, altered DA function in either the striatal punches or the media beyond that induced by exposure to only the PCB mixture.

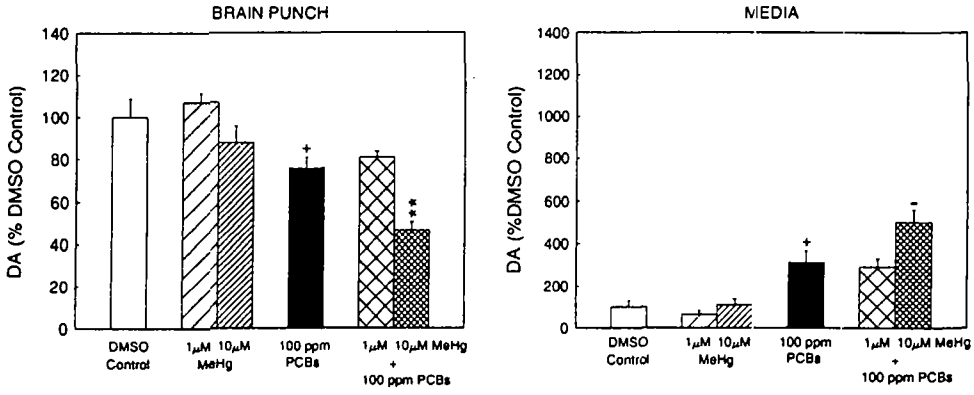

Figure 1. Effects of methylmercury (MeHg) alone or in combination with PCBs on striatal punch and media concentrations of dopamine (DA). +$p\leq0.05$ with respect to DMSO control; -$p\leq0.1$, **$p\leq0.01$ with respect to 100 ppm PCBs.

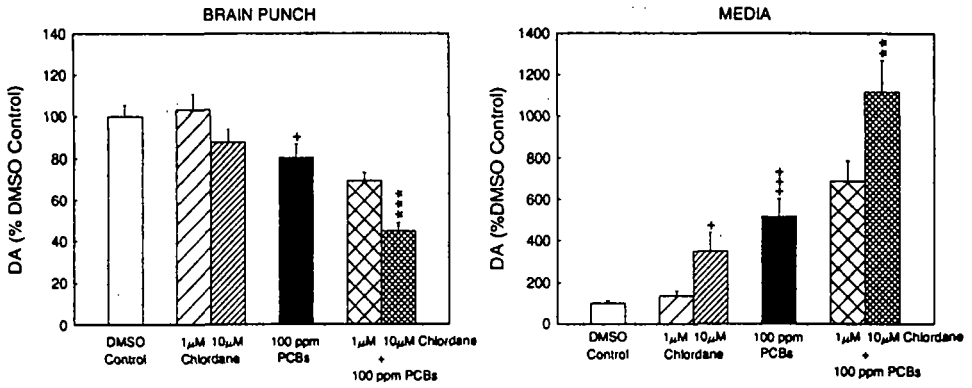

Figure 2. Effects of chlordane alone or in combination with PCBs on striatal punch and media concentrations of dopamine (DA). $+p \leq 0.05$, $+++p \leq 0.001$ with respect to DMSO control; $^{**}p \leq 0.01$, $^{***}p \leq 0.001$ with respect to 100 ppm PCBs.

These results confirm that acute, *in-vitro* exposure of striatal tissue from adult rats to a mixture of PCBs similar in their congener makeup to the congeners found in GL salmon alters DA function — reducing tissue concentrations of DA and elevating media concentrations of DA. These findings are similar to those we have recently reported using striatal slices (5). The reductions in tissue concentrations of DA cannot be explained as being due to the increased media concentrations and appear to be due to inhibition of DA synthesis at the level of the rate limiting synthetic enzyme, tyrosine hydroxylase (TH) (6). The PCB-induced elevations in media DA concentrations are similar to those we and others have reported (5,7) and are due either to enhanced release or to PCB-induced inhibition of the high affinity DA transporter that removes DA from the synaptic cleft. Either increased DA concentrations in the synaptic cleft or exposure to the DA auto-receptor agonist, apomorphine may activate the presynaptic DA auto-receptor resulting in alterations in the phosphorylation state of TH or its affinity for the cofactor, tetrahydrobiopterin and hence, reductions in the synthesis of DA (8).

Perhaps more importantly for this series of experiments, we have demonstrated that co-exposure of striatal punches to PCBs and either MeHg or chlordane results in significant alterations in the above described DA measures that are significantly greater than seen following exposure to the singly presented contaminants. Although the mechanisms responsible for these interactions have not been fully elucidated, the similarities of action of PCBs+MeHg and PCBs+chlordane suggest possible common sites of action.

Exposure of cerebellar microsomes (9) to PCBs results in increased intracellular concentrations of calcium, due to activation of the ryanodine channel. Since MeHg also elevates intracellular calcium concentrations, albeit via an inositol-1,4,5-tris-phosphate (IP_3) sensitive channel (10), it is possible that the combined exposure of the punches to PCBs+MeHg results in complementary increases in intracellular calcium which in turn may alter intracellular communication pathways and the synthesis and release of DA. Indeed, Kalish and Racz (11) report that exposure of mouse striatal slices to 50 or 100 μM concentrations of MeHg (levels higher than used in this experiment) result in a concentration

dependent increase in the spontaneous efflux of DA while Faro *et al.* (12), using *in-vivo* microdialysis, reported increased release of DA following exposure to MeHg. Thus, both PCBs as well as MeHg enhance the endogenous release of DA which, as we previously mentioned, may alter the synthesis of DA via activation of inhibitory DA auto-receptors.

There is less available data on the actions of chlorinated pesticides on the synthesis and release of DA. Chlorinated pesticides have been shown to alter both DA binding and uptake in rat brain synaptosomes (13). In addition, both PCBs (14) and chlordane (15) inhibit Na^+/K^+- and Mg^{2+}ATPase activity resulting in decreased cellular metabolism. Decreased Na^+/K^+ATPase activity has been shown to be selectively neurotoxic in rat brain (16).

In summary, the above results clearly demonstrate that combined exposure of striatal punches from adult rats to PCBs and either MeHg or chlordane result in a greater inhibition of punch DA concentrations and elevations in media DA concentrations than seen following exposure to the contaminants when presented singly. These findings illustrate the importance of determining the possible interactions between the toxicants found in contaminated fish since fish consumption guidelines and risk assessments based on the actions and levels of a single component of a complex mixture of contaminants will result in significant underestimation of the risk associated with consumption of the contaminated food stuffs.

Acknowledgements

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Note by Richard F. Seegal:

We have been unable to replicate the findings- that the hydroxy metabolite of 3,4,3',4'-TCB decreases brain concentrations of L-DOPA - presented in the abstract `Estrogen-Like Neurochemical and Reproductive Effects of the Major Metabolite of 3,4,3',4'-tetrachlorobiphenyl (3,5,3',4''-tetrachloro-4-biphenylol)'.
These results were presented at the Dioxin '97 meeting in Indianapolis (*Organohalogen Compounds, 4*, pp 125-128). We wish to withdraw this abstract.