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Exposure of Nervous System Cells to Polychlorinated Biphenyls (PCBs) Results in Significant Alterations in Levels of Expression of Neurotrophic Factors

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Toxic insult by PCBs results in learning and memory deficits in humans. Alterations in expression of neurotrophic factors and/or their receptors have been linked to changes in cognition. How PCBs affect cognition is not known. We propose that PCBs affect cognition by altering neurotrophic factor expression or effects. We exposed cultured C6 glial cells in medium containing PCB (Aroclor 1254 (10ppm)). Control cells were treated with DMSO or regular medium. Cells were incubated at 37º C for up to 72 hours. Medium samples were taken at 6hr, 24hr, 48hr, and 72hr intervals. Enzyme-linked immunosorbent assays (ELISA) were used to determine both glial cell-line derived neurotrophic growth factor (GDNF) and nerve growth factor (NGF) concentrations in all samples. In addition, we removed the brain from rats exposed to10ppm, 50ppm, and 0ppm PCB in their diet for 7 or 84 days. One cerebral hemisphere from each animal was homogenized and analysized by ELISA for GDNF and NGF content. PCB increased GDNF but not NGF expression in Glial cells. Contrary to our in-vitro data, PCB treated rats had significantly reduced GDNF and NGF in their brains. Our data show that exposure of neural tissues to PCBs, alters NGF and GDNF expressions and hence offers a basis whereby PCB may alter neural plasticity.

Introduction

Neurotrophic factors (NFs) are substances important for the growth of neurons and their phenotypic expression during development. Most neurons require a sustained supply of these trophic factors throughout their lifetime for their maintenance and recovery from nervous system injury in the adult organism. In addition, neurotrophic factors have also been shown to be important regulators of learning and memory. Results of recent studies suggest that blockade of NGF effects in the brain results in impaired learning performance (Van der Zee et al., 1995), while administration of NGF enhances learning performance. (Lipinski et al., 1995). Studies in gene knockout mice indicate that low levels of GDNF in the central nervous system is associated with impaired learning performance. Results of other studies have demonstrated that acquisition of long-term potentiation and spatial learning are associated with elevated expression of brain-derived neurotrophic factor (BDNF).

Given the important roles NFs play in learning and memory, this study is concerned with the altering-potential of toxic substances such as PCBs on the expression of these NFs and their receptors. The nervous system has been shown to be highly sensitive to the effects of PCBs. Epidemiological studies in humans have demonstrated a variety of nervous system deficits associated with PCB exposure, including alterations in function of the peripheral nervous system and central nervous system (China and Chu, 1984). Impairments in learning and memory have been documented both in young and older humans (Schantz et al., 2001) following environmental exposure to PCBs, and in rats exposed to PCBs. Although PCB exposure has been shown to alter a variety of intracellular signalling processes in neurons, very little is known concerning the mechanism by which PCBs affect learning and memory. Furthermore, very little is known concerning effects of PCBs on neurotrophic factor expression or effects (Angus and Contreras, 1994). We propose that PCBs affect cognition by altering neurotrophic factor expression or effects

Hypothesis/Specific Aim

Hypothesis: Exposure of nervous system cells to PCBs results in significant alterations of neurothrophic factor expression.

Aims:

1. ***To determine the acute effects of PCBs on glial cells (the most abundant cell type of the brain) through in-vitro studies.***
2. ***To determine both acute and chronic effects on whole brain tissue through in-vivo studies.***

Methods

Culturing: We cultured C6 cells at passage 39 in100mm plates in 10 ml high glucose DMEM + 10% FBS per dish. Cultures were incubated at 37 ºC with medium changes of every two days until 60 – 70 % confluence was achieved. Cells were lifted with 10% trypsin/EDTA in Calcium Magnesium-free Tyrodes and resuspended in medium. Cells were plated at a constant seeding concentration of 300 mL cell suspension in 3 ml medium in 60 mm culture dishes. A minimum of 3 plates for each of four intervals per treatment—6, 24, 48 and 72 hours were standard for each experiment. All *in-vitro* experiments were repeated a minimum of five times.

Treatment and sampling: At 100% confluence, medium was withdrawn and replaced by medium containing PCB in DMSO (10 ppm) DMSO (10 ppm) or normal medium. Each plate was loaded with exactly 2 ml of medium, twelve plates per treatment, with 3 plates per time interval.

Sampling: plates were gently swirled clockwise and anti-clockwise with care to avoid dislodging of monolayer. One – two ml of medium was sampled from each plate, and stored at – 20 ºC.

Methods continued

Live-dead determination: To ensure that the results are not influenced by toxic induced apoptosis, we did live-dead assays using **calcien** to stain healthy cells (green) and **Ethd-1** to stain dead cells (red). Cells from several randomly select visual fields were counted and live/dead ratio determined.

In-vivo: A minimum of 4 rats per treatment were exposed to10ppm and 50ppm and 0ppm PCB in their diets. Two temporal groups were treated, one for a week the other for 3 months. Animals were sacrificed by CO_2 euthanization. Brains were removed after decapitation and separated into hemispheres. The brains were instantly frozen in dry-ice-cold 2 methyl butane and stored at -20 ºC

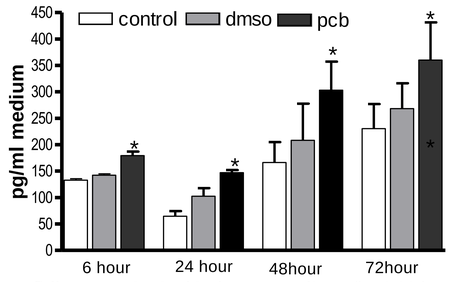
Brain processing: One hemisphere from each brain was dipped in liquid N and then crushed by hammering The resultant powder was then homogenized in 4ml of processing buffer while kept on ice to minimize protein degradation. Content was ultra centrifuged at 4 ºC and 2400 rpm for 30 min and supernatant was stored at -20 ºC.

Data collection: Enzyme-linked immunosorbant assay (ELISA) determined GDNF and NGF concentration in both cell culture and brain samples. For cell culture, samples from three different culture plates for each treatment and time interval were loaded in 96 well ELISA plates in quadruplicate. Averages from the four readings per plate as well as the three plates per treatment were calculated to determine the average concentration per treatment for each time point. Brains supernatant were loaded in quadruplicate per animal as above.

Statistic tools: The Student's T test and Dunnett's Post Hoc was used to determine differences among treatments. Significance was accepted for P = < 0.05 with SD 1 from the mean.

Results

Fig.1 Treatment with PCB 10ppm caused significant increase in GDNF secretion for all time points.



Cells were maintained in the same medium of an initial inoculation of 10ppm PCB (Aroclor 1254) N= 5 with triplicate plates. '*' indicates P value < 0.05 Student's 2-tailed T test.

Fig. 2 There was a general change in GDNF production for all treatments over time, where there was a reduction between the first 6hr and 24 hr, there after increasing

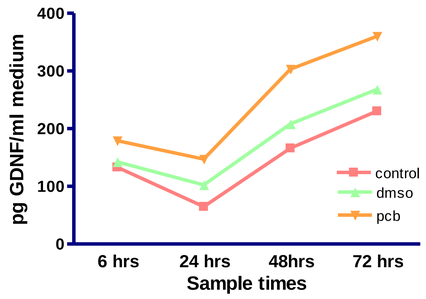


Fig. 3 Treatment with PCB 10ppm Showed similar effects on NGF expression as on GDNF in fig. 1, where NGF seem to be increased by PCB compared to DMSO but the differences were not statistically significant among treatments.

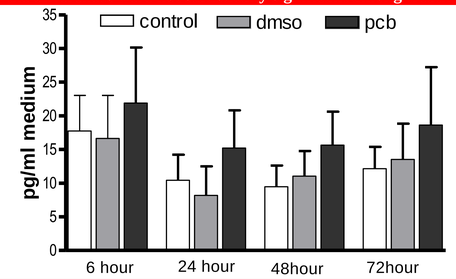


Fig. 4 Results of live/dead cells from several randomly selected visual fields indicate no significant difference in apoptosis of treated to control cells (actual data not shown). Below are examples of two typical fields. Each paired Photos are from the same field, achieved by using two different fluorescence filters.

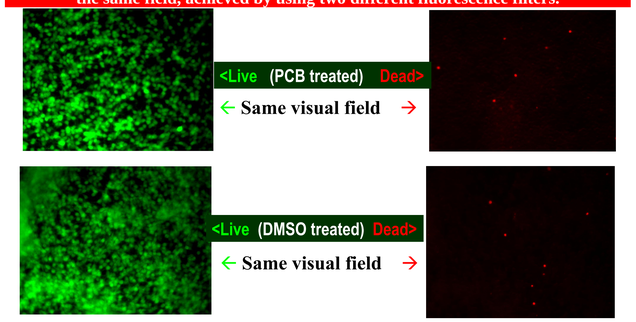
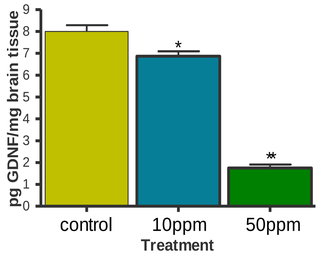
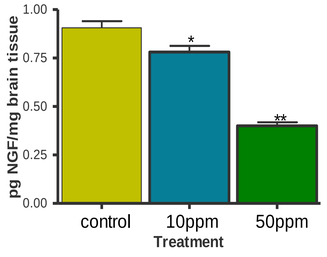


Fig. 5 Rats fed PCB inoculated pellets for 7 days showed significant dose related GDNF reduction in their brains



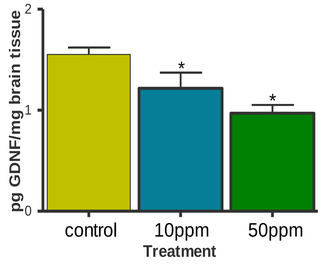
** P<0.001, *P<0.05 compared to controls. N = 4

Fig. 6 Rats fed PCB inoculated pellets for 7 days showed significant dose related NGF reduction in their brains



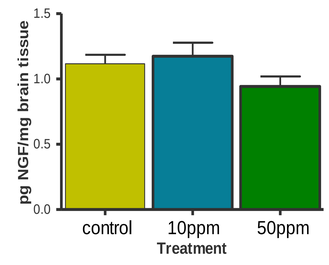
** P<0.001, *P<0.05 compared to controls. N = 4

Fig. 7 Rats fed PCB inoculated pellets for 84 days showed significant dose related GDNF reduction in their brains



*P<0.05 compared to controls. N = 4

Fig. 8 Rats fed PCB inoculated pellets for 84 days showed no significant difference in NGF expression



*P<0.05 compared to controls. N = 4

Summary

• PCB induced increased GDNF expression in cultured glial cells. We think that this may be an initial protective measure which diminishes with time once the insult is sustained. This can be expected since glial cells also have a protective function.

•Glial cells excrete NF (GDNF and NGF) in culture. Interestingly, in the study there is overall reduction from the 6hr time point to the 24hr and increase after for all treatments. This may suggest reuptake of NF during the first 6 hr and accumulations in the medium there after, due to continuous secretion.

•Although there seem to be a similar trend in NGF expression as GDNF, the differences were not statistically significant. This may be attributed to the high variability among the groups.

•Neither PCB nor DMSO caused significantly different apoptosis in cultured C6 cells when compared to control.

• Interestingly in-vivo studies indicate that PCB is a potent reducer of both GDNF and NGF concentration in rat brains over short term toxic insult. The in-vitro/in-vivo discrepancy may be expected since NF's analysis was based on gross extraction from whole brain including all the cell sub-types. More studies are needed to determine effects at discrete brain location of interest like the hippocampus.

• The effect of PCBs on NF expression is dose dependent but seem to be less severe with chronic or long term exposure in nervous system cells. In this study the effect on NGF disappeared after 84 days of exposure. This may be indicative of adaptation.

Conclusion

Apart from their role in necessitating growth, development and maintenance of neurons, neurothrophic factors (NFs) also promote neural plasticity and hence learning and memory. Substances with the propensity to alter these factors and/or effects may in doing so also alter cognition. It has been established that PCBs impair cognition in both humans and animals. We wanted to determine whether PCBs alter NFs and that, consequently be a mechanism by which they affect cognition. From this study there is strong evidence that PCB (Aroclor 1254) at a concentration as low as 10ppm, do significantly alter the expression of GDNF and NGF in nervous system cells, both *in-vitro* and *in-vivo*. Such findings may provide evidence that PCBs and other neural toxins affect learning and memory at least partly by their ability to alter NF..

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Acknowledgements

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