POLYHALOGENATED HYDROCARBON INDUCED PERTURBATION OF INTRACELLULAR CALCIUM HOMEOSTASIS: FROM ASTROCYTES TO HUMAN MACROPHAGES.

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Introduction

Polychlorinated biphenyls (PCB's) are persistent in the environment causing adverse effects in many organisms. Although the use of these chemicals was banned as early as 1978 their persistent nature is of concern for human health especially during foetal development. PCB's are abundant as mixtures of over two hundred congeners and are found in human milk and brain. The coplanar congener type binds to the arylhydrocarbon (Ah)-receptor which is thought to be the cause for its dioxin-like mode of action. The non-planar congeners do not bind to the Ah-receptor, however they interfere with a couple of receptors, enzymes and with intracellular calcium ($[Ca^{++}]_i$) regulation [1, 2, 3].

In analogy to the persistent PCB compounds polybrominated biphenylethers (PBDE's) are of growing concern with regard to human health because they are used in large quantities as flame retardants in a variety of applications. Their environmental distribution seems to be similar to that of PCB's and DDT. They were also found, because of their lipophylicity, in human milk with the tendency of an exponential increase of tissue concentrations during the last 3 decades [4]. In contrast to the meanwhile elaborated knowledge about the mechanisms of action of PCB's there is almost no exact information about PBDE toxicity, especially as neurotoxicity is concerned.

Methods and Materials

2,2',4,4'-tetrachlorobiphenyl (PCB47), 3,3',4,4'-tetrachlorobiphenyl (PCB77) and 2,2', 4,4',5-pentabromodiphenylether (PBDE99) were purchased from Promochem, (Wesel, Germany). The procedures of culturing human macrophages, rat astrocytes, rat neurones, bovine chromaffin cells from the adrenal medulla as well as rat PC-12 cells have been described [5, 6, 7, 8, 9]. Microspectrofluorometry of $[Ca^{++}]_i$ in single cells was performed using the Fura-2/AM method in a PTI (Lawrenceville, NJ, USA) video imaging system [8].

Results and Discussion

Incubation of human macrophages with PCB47 using either short term treatment or 5 hrs exposure did not change the basal $[Ca^{++}]_i$ levels of unstimulated cells. ATP stimulation induced a concentration dependent peak-like elevation of $[Ca^{++}]_i$ which showed desensitation and could be blocked by application of ATP receptor antagonists. Due to 5hrs PCB47 incubation the concentration effect relationship of ATP versus $[Ca^{++}]_i$ was drastically shifted to the left. The shape of the concentration effect relationship after PCB treatment showed evidences for a shift in ATP receptor types from two to one. This PCB47 induced perturbation of ATP stimulation mediated $[Ca^{++}]_i$ level elevations depended on the concentration in a range between 5 µM and 100 µM PCB47. Experiments using calcium deficient media revealed that the extracellular calcium

contributed the overwhelming part of PCB47 induced elevation of $[Ca^{++}]_i$ levels. A similar left shift of the ATP versus $[Ca^{++}]_i$ concentration effect relationship was induced by inhibition of the NO synthase, a procedure which itself blocked the capability of PCB47 to shift the ATP/$[Ca^{++}]_i$ concentration effect relationship to the left. Moreover, 5 hrs PCB47 incubation reduced cAMP levels of unstimulated cells in a concentration dependent manner in a range between 10 nM and 10 μM PCB47. In addition the release by macrophages of TNFα was compromised by PCB47 treatment.

In cultured rat astrocytes as well as in bovine chromaffin cells from the adrenal medulla acute PCB47 treatment induced elevations of $[Ca^{++}]_i$ levels after 15-20 min treatment. Experiments using calcium deficient media evidenced that influx of calcium from the extracellular space primarily contributed to this phenomenon. In cultures of astrocytes as well as PC-12 cells from rats PBDE99 induced similar perturbations of $[Ca^{++}]_i$ levels. Depending on the cell type elevations of basal levels as well as peak-like oscillations could be observed in concentration ranges lower than 10 μM.

The present results show that in different mamalian cell types ranging from excitable cells like neurones to non-excitable cells like macrophages non-planar PCB congeners as well as PBDE's disrupt the homeostasis of $[Ca^{++}]_i$ levels. A main point of this investigation is that in human macrophages as well as in rat astrocytes and bovine chromaffin cells this phenomenon could exclusively be shown for the non-planar congener PCB47 whereas the coplanar sister-congener PCB77 was completely uneffective. The induce by non-planar PCB's and PBDE's of elevating the $[Ca^{++}]_i$ level may have dramatic consequences for signal transduction pathways within the cells:

1. Calcium itself is one of the main second messengers mediating important cellular functions.
2. The interference by these congeners with the levels of cyclic nucleotides as shown in human macrophages shows that important second messenger pathways may be incorporated into the dysregulation of cellular function.

It has been shown recently by Shafer's group [7,10,11] that in cultivated developing cortical neurones a PCB mixture mostly containing non-planar PCB congeners, i.e. Aroclor 1254, produced a peak-like elevation of $[Ca^{++}]_i$ levels upon acute exposure. This $[Ca^{++}]_i$ peak was followed (after recovery of $[Ca^{++}]_i$ to basic levels) by a slowly developing increase of the basal $[Ca^{++}]_i$ level. This sometimes was superimposed by concentration dependent peak-like calcium oscillations which could be blocked by dihydropyridine like calcium channel antagonist. Also in this case extracellular calcium contributed the most part of $[Ca^{++}]_i$ level elevations presumably via voltage operated calcium channels as well as GABA regulated receptor channels as was shown by the application of calcium channel blockers and GABA receptor agonists. The phosphorylation of cyclic nucleotide responsive element binding sites (CREB) was increased in these neurones due to this treatment with the primarily non-planar congener containing PCB mixture Aroclor 1254. These experiments in developing neurones in vitro contribute important evidences that besides changes in membrane receptor and ion channel function basic capabilities of the cell nucleus, i.e. DNA-binding, and transcription/translation are interfered with which might contribute to cellular dysfunction produced by PCB's.

With regard to the toxicity of PCB's and especially its risk assessment using the toxic equivalent factor (TEF) concept the results presented here show evidences from a variety of mammalian cells (including human ones) that using TEF's for the estimation of non-planar PCB congener toxicity may fail its purpose because the described actions exclusively were due to treatments using congeners without Ah-receptor binding capacity.

Acknowledgements
Part of this work was funded by DFG (IIIGK-913-98) and IC (QLRT-1999-01562).

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