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Repeated exposure of adult rats to Aroclor 1254 induces neuronal injury and impairs the neurochemical manifestations of the NMDA receptor-mediated intracellular signaling in the hippocampus

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ABSTRACT

Aroclor 1254 is a mixture of polychlorinated biphenyls (PCBs), a class of environmental toxins which cause a wide spectrum of neurotoxic effects. Learning and memory deficits are the profound effects of PCBs which may be related to hippocampal dysfunction. To get insight into the underlying neurochemical mechanisms, we employed the microdialysis technique to investigate the effect of repeated exposure of adult male Wistar rats to Aroclor 1254 (10 mg/kg b.w., daily, ig., for 14 days), on the neurochemical parameters of NMDA receptor-mediated glutamatergic signaling in the hippocampus in vivo assessed using the microdialysis technique. The results demonstrated that exposure to Aroclor 1254, which was associated with substantial neuronal damage and loss in the hippocampus, markedly decreased the NMDA-induced extracellular accumulation of newly loaded $^{45}CaCl_2$, cGMP and glutamate, and reduced the basal content of the NO precursor, arginine, indicating inhibition of the NMDA/NO/cGMP pathway. Aroclor 1254 exposure also decreased the basal microdialysate content of glutamate and glutamine, which may cause inadequate supply of the neurotransmitter glutamate, while the level of two other neuroactive amino acids, aspartate or taurine was not affected by the exposure. The results underscore neuronal lesion and inhibition of NMDA receptor-mediated glutamatergic signaling in hippocampus as a potential major contributor to the cognitive deficits associated with exposure to PCB.

1. Introduction

Polychlorinated biphenyls (PCBs) are environmental toxins which avidly accumulate in mammalian tissues, including the brain. Multiple studies in occupationally exposed humans and in animal models have documented an array of neurophysiological effects of PCBs including cognitive and motor dysfunctions, which are either evoked in adults upon direct exposure (Nishida et al., 1997; Donahue et al., 2004; Peper et al., 2005; Lin et al., 2008), or present as delayed psychomotoric development upon prenatal or early postnatal exposure (Gilbert and Crofton, 1999; Llansola et al., 2009; Piedrafita et al., 2008; Park et al., 2010; Boix et al., 2011). Studies with animal models in both the pre- and postnatal setting and examination of the effects produced in neurons cultured in vitro revealed an array of neurochemical changes indicative of PCB-induced neuronal damage (Tilson and Kodavanti, 1997; Mariussen et al., 2002; Fonnum and Mariussen, 2009). The changes include, among others, accumulation of reactive oxygen species (ROS), which may be triggered by overactivation of the NMDA class of glutamate receptors, leading to oxidative/nitrosative stress and glutamatergic dysfunction, and to deregulation of intracellular signaling pathways (Mariussen et al., 2002; Fonnum and Mariussen, 2009). Considerable evidence suggests that PCBs also target other neurotransmitter systems in different brain regions: the dopaminergic (Seegal et al., 1991; Yun et al., 2005), acetylcholinergic (Donahue et al., 2004) and serotoninergic system (Khan and Thomas, 2004).

Links between the alterations of neurotransmitter signaling and/or oxidative tissue damage and the particular neurophysiologic or neurobehavioral consequences of exposure to PCBs have not been examined in much detail. Cognition, memory and learning, the neurophysiologic modalities which are frequently affected by PCBs (Donahue et al., 2004; Gilbert and Liang, 1998; Schantz et al., 2001), are in a substantial degree controlled by the hippocampus (Lynch, 2004). In the hippocampus PCBs disrupted actin cytoskeleton which is a morphological carrier of synaptic

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plasticity (Tang et al., 2007), and affects synaptic transmission (Hong et al., 1998; Niemi et al., 1998), including long-term potentiation (LTP), the neurophysiological basis of memory formation (Gilbert and Liang, 1998; Gilbert and Crofton, 1999; Ozcan et al., 2004). Since LTP depends on the activation of synaptic N-methyl-D-aspartate receptors (Zhuo et al., 1994; Lynch, 2004), we endeavored to analyze changes in the different markers characterizing events downstream NMDA receptor activation, in hippocampus of rats which were administered Aroclor 1254, a commercially available mixture of PCB congeners, for 2 weeks. The key events triggered by NMDA receptor ligands are massive influx of Ca^{2+} to neurons *via* NMDA receptor channels, triggering also a phenomenon of calcium induced calcium release (CICR) from the ER stores (Lazarewicz et al., 1998; Makarewicz et al., 2000; Salinska et al., 2000). This is followed by nitric oxide synthase (NOS) activation, generation of nitric oxide (NO), activation of guanylate cyclase and production of cGMP. NO and cGMP are the molecules critical for the regulation of synaptic plasticity, learning, and other complex behaviors (Lynch, 2004, and references therein).

After having established that Aroclor 1254 treatment in this model induces considerable neuronal damage in the hippocampus, we employed the brain microdialysis technique to evaluate the NMDA induced release of ^{45}Ca, which reflects sequential generation of the Ca^{2+} signal by the NMDA receptors and ryanodine receptors located on the rough endoplasmic reticulum (Lazarewicz et al., 1998; Makarewicz et al., 2000, and references therein), and of cGMP, which in addition to being the critical carrier of the signal triggered by NMDA receptor activation serves as a convenient marker of the NMDA/NO/cGMP signaling pathway activity (Hermenegildo et al., 2000; Hilgier et al., 2003). We also compared the effects of Aroclor 1254 treatment on the basal and NMDA-evoked extracellular accumulation of glutamate and glutamine, parameters reflecting the availability of glutamate for interaction with the NMDA receptor. To assess the neurotransmitter system-specificity of the changes we also studied the extracellular accumulation of other neuroactive amino acids: aspartate and taurine.

2. Material and methods

2.1. Reagents

Aroclor 1254 was purchased by LGC Standards Poland (Lomianki, Poland). All other chemicals were of analytical grade and were obtained from Sigma–Aldrich Poland (Poznan, Poland). $^{45}CaCl_2$ was obtained from the Polatom (Swierk, Poland). The cGMP assay kit (Enzymeimmunoassay Biotrak (EIA) System) was from Amersham Biosciences, Bristol, UK.

2.2. Animals, administration of Aroclor 1254

Young adult male Wistar rats weighing 200–250 g were used in this study. All animal experiments were carried out in accordance with domestic regulations and the European Community Council Directive of 24 November 1986 (86/609/EEC). Experimental protocols were approved by the local ethical committee in Warsaw. Aroclor 1254 dissolved in the volume of 200 μl of rape oil, was administered *via* the gastric tube in the dose of 10 mg/kg b.w. once a day for 14 days. The rats from the control group received the same volumes of the vehicle.

2.3. Light microscopy and counting of neurons

The animals were anaesthetized with Nembutal (80 mg/kg b.w.) and perfused through the ascending aorta with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 20 °C. The sampled tissue (CA1 region of the hippocampus) was fixed in the same solution for 20 h. Semi-thick sections (5 μm) were then stained using the routine toluidine blue method and examined in light microscopy. The toludine blue-stained neurons were counted in 10–12 different fields of the sections, each obtained two control and two Aroclor 1254-treated rats.

2.4. Transmission electron microscopy

Tissue perfusion was performed and tissue samples (CA1 region of the hippocampus and forebrain cortex) were fixed as described under Section 2.3. Thereafter samples were rinsed in cacodylate buffer (pH 7.4) and postfixed in a mixture of 1% OsO_4 and 0.8% $K_4[Fe(CN)_6]$ for 2 h. The material was then dehydrated in graded ethanol and propylene dioxide and embedded in Spurr. Ultrathin sections were analyzed in a JEM-1200EX.

2.5. Microdialysis of rat hippocampus

Microdialysis probes CMA/11 (CMA Microdialysis AB, Solna, Sweden), membrane length 1 mm and outer diameter 0.24 mm, were used. They were implanted stereotaxically into the hippocampal formation of rats anesthetized with urethane (1.25 g/kg intraperitoneally). The coordinates for the tip of the cannula 2.3 mm lateral and 3.8 mm posterior relative to the bregma and 3.5 mm ventral from the *dura mater*, were established according to the stereotaxic atlas of Paxinos and Watson (1982), with the incision bar in the stereotaxic frame set at −3.3 mm. For perfusion of the probes two different buffers were used. In the ^{45}Ca efflux experiments, perfusion was done with Krebs Ringer bicarbonate (KRB) buffer was used containing 122 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.4 mM KH_2PO_4 and 25 mM $NaHCO_3$ (pH 7.4), the buffer used in experiments measuring cGMP or amino acid contents was the artificial cerebrospinal fluid (ACSF) containing (in mM): Na^+ 150; K^+ 3.0; Ca^{2+} 1.2, Mg^{2+} 0.8; H_2PO_4 31.0; Cl^- 155; pH 7.4. The rate of dialysis was 2.5 μl/min.

2.6. Detection of ^{45}Ca efflux

Immediately after implantation, the probes were perfused for 1 h with KRB medium supplemented with 25 μCi of $^{45}CaCl_2$, which was followed by 150 min equilibration-perfusion with nonradioactive KRB medium to wash out the excess of the label. Then samples of dialysate were collected every 5 min. After 30 min of measuring the control efflux, the medium containing 10 mM NMDA was introduced for 20 min, followed by return to a control dialysis medium. The radioactivity of ^{45}Ca in each sample of dialysate was measured by liquid scintillation counting using the Wallac 1409 beta counter (Wallac Oy, Turku, Finland). Changes in ^{45}Ca efflux were calculated as described previously (Lazarewicz et al., 1998). A steady state decay in ^{45}Ca radioactivity in dialysates (a straight line on the semilog plot vs. time), which was reached after 2.5 h of washing, was calculated for each individual experiment. This basal efflux level was used to calculate the % changes in ^{45}Ca efflux evoked by NMDA.

2.7. Determination of cGMP and amino acids in the microdialysates

Samples of probes perfused with ACSF were collected every 40 min in the period of 40–240 min of perfusion, and 1 mM NMDA was infused for 40 min as indicated. cGMP was determined with cGMP EIA System (Amersham Biosciences, Bristol, UK) according to the manufacturer's protocol. Amino acids were analyzed using HPLC with fluorescence detection after derivatisation in a timed reaction with o-phthalaldehyde (OPA) plus mercaptoethanol, exactly as described earlier (Hilgier et al., 1999).