Dietary Exposure to the PCB Mixture Aroclor 1254 May Compromise Osmoregulation by Altering Central Vasopressin Release

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Introduction

Despite the importance of systemic osmoregulation, the potential deleterious effects of persistent organochlorines, such as polychlorinated biphenyls (PCBs), on body fluid regulation has not been thoroughly investigated. In an effort to ameliorate this deficit, the current study explores the toxic effects of PCBs on osmoregulation, and in particular, on the activity of the magnocellular neuroendocrine cell (MNC) system of the hypothalamus. MNCs of the supraoptic nucleus (SON) release oxytocin (OXY) and vasopressin (VP) from terminals in the neurohypophysis in response to dehydration. The latter is released to effect water conservation in response to dehydration via its action upon the kidney and through extra-renal actions[1]. MNCs also secrete VP from their cell bodies and dendrites locally i.e., into the extracellular space of the SON. Although it has been shown that both intranuclear and systemic release rise in response to dehydration the physiological significance of intranuclear release has not been fully elucidated[2].

Several lines of evidence suggest that intranuclear VP may act as a short-loop feedback to modulate systemic VP release: 1) By regularizing the firing pattern of MNCs within the SON, VP promotes firing patterns[3] that are consistent with efficient systemic VP release[4] 2) VP can also depress MNC firing activity by reducing the presynaptic activity of excitatory inputs impinging upon them[5,6]. 3) Blockade of central VP receptors can exaggerate dehydration-induced VP release systemically[7]. In addition, data from our lab suggests that endogenous VP acts on autoreceptors in the SON to further augment central (SON) VP release[8]. Collectively, these findings suggest that intranuclear VP may negatively modulate systemic VP release, potentially restraining excessive VP release during increased physiological demand[9,10]. We have chosen to investigate the MNC system as a potential target of PCBs because these cells are among the most highly vascularized in the brain, and their projections are not protected by the blood brain barrier[11], increasing the likelihood of accumulation of toxins in these cells.

There are 209 different conformations of PCBs, based on the number and position of chlorine substitutions. PCBs were formerly used as electric fluids in transformers and capacitors as well as heat transfer fluids and hydraulic lubricants, and some of these applications resulted in a direct or indirect release of PCBs into the environment[12]. Synthetic organochlorines such as

dioxins and PCBs are perfect candidates for worldwide pollution because they ignore virtually all boundaries, natural and artificial. The high lipophilicity and chemical stability of PCBs have resulted in widespread environmental contamination and accumulation in both the biotic and abiotic environment. Following environmental exposure, PCBs selectively accumulate in lipid-rich tissues such as brain[22], adipose tissue and breast milk[13]. PCB mixtures are sufficiently potent toxins in animal models and human health effects are evident even at background exposure levels[14]. An abundance of evidence indicates that PCBs are classic persistent neurotoxins. For example, PCB exposure can produce subtle CNS effects resulting in deficits in learning and memory[15,16]. Developmental neurotoxicity has been reported in humans[17,18] and may be implicated in the etiology of learning/cognitive disorders such as autism. PCBs are also known endocrine disrupters[19]. Humans are exposed to PCBs primarily through ingestion, and the Aroclor 1254 profile is detected at significant levels in several common supermarket foods[20,21]. In light of our intent to achieve *in vivo* brain levels that may be comparable to human or animal brains potentially exposed to environmental PCBs over a lifetime, the dosing regimen chosen for these initial studies in the rat (30mg/kg/day;15 days) is based on previous reports that brain PCB levels achieved using a similar dosing regimen (30mg/kg/day for 28 days yielded 8.0-15.0 ppm or 30-50 μM[22]) are on a similar order of magnitude to those found in wildlife brains[23]. These tissue concentrations have been shown to disrupt neuronal signaling without producing overt cell death[24,25].

We chose to use voluntary ingestion as the route of PCB exposure since it is more reflective of natural exposure compared to ip injection. One unexpected observation that resulted from pilot studies using ip injection of PCBs was the deleterious effects of the vehicle (corn oil) resulting in pooling of lipid within the abdominal cavity, mottling of the liver, fatty liver and general discoloration of all abdominal viscera at time of sacrifice. Therefore, all work described in this series of experiments have employed voluntary ingestion of the toxin. Work described in this paper suggests that PCBs in concentrations reflecting realistic lifetime exposure levels may negatively impact homeostatic mechanisms responsible for body water balance by altering somatodendritic (intranuclear) VP secretion in response to dehydration in vivo. The downstream consequences of such influence is currently under investigation, and preliminary evidence suggests that the final output of the MNC system may be greatly exaggerated during physiological stress in PCB-exposed rats.

Methods and Materials

Animals and Tissue Harvest: Adult male Sprague-Dawley rats were individually housed with food and water provided *ad libitum*. Animals were weighed daily for accurate dosing at 30mg/kg/day for 15 days. To overcome expected neophobia to the delivery treat, animals were subjected to a 7 day pre-conditioning period to Cheetoh© cheese puffs as daily supplements to their regular diet. On the 1st day of exposure to PCBs, Aroclor 1254 suspended in corn oil vehicle was injected into a Cheetoh© cheese puff. The PCB-laced cheetoh was immediately delivered and voluntarily consumed in its entirety within 3-5 minutes. On the 15th day of the study, animals were injected intraperitoneally (ip) with 0.6 ml/kg of either 3.5 M or 0.9% NaCl and water was withheld until sacrifice 4-4.5 hrs later, during which time SON VP release has been shown to be elevated following a hyperosmotic ip injection[2]. Tail blood was collected and plasma osmolality measured just before sacrifice to confirm dehydration state and match the osmolality of aCSF (Locke's solution) used to bathe the brain tissue *in vitro*. After decapitation, brains were removed and each SON was maintained in an individual chamber at 37°C. Control samples (from 0.9% saline-

injected rats) were incubated in normal aCSF (300 mOsm) prepared as reported in[5]. Stimulated SON samples (from 3.5 M saline-injected rats) were incubated in aCSF to which NaCl had been added to match plasma osmolality (310-350 mOsm). Each chamber contained unilateral SON from one half of each brain per rat in a total volume of 500 µl oxygenated aCSF[5].

In Vitro Preparation: SON tissue was incubated for a 10-min experimental period, after which aliquots of perfusate were removed and frozen for subsequent VP analysis. Tissue sample was collected in cold protease inhibitor cocktail, homogenized and frozen for later protein determination as previously described[5]. VP values for each sample were normalized to the total protein present in each SON sample to control for variations in the size of the SON punches.

Quantification of VP: Plasma samples were first delipidated and plasma VP extracted using acetone-petroleum ether[26] and subsequently analysed with a sensitivity of 3.39 pg/ml using enzyme-immunoassay (arg8-vasopressin correlate EIA kit, Assay Designs) as previously described[5]. Vasopressin values were corrected for dilution and expressed as pg/ml. For SON perfusates, values were normalized to total protein in the SON and expressed as pg/ml/µg protein.

Statistical Analysis: VP values were analyzed for main effects of PCB and/or hydration state by one-way or 2-way ANOVAs on Sigma Stat software. Where overall significance of ($p<0.05$) was obtained, *post hoc* multiple comparisons were used to detect specific group differences.

RESULTS AND DISCUSSION

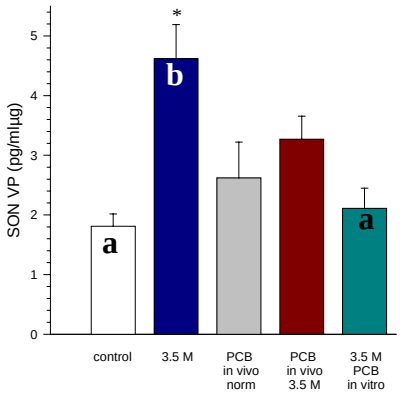


Figure 1. SON vasopressin release in response to dietary exposure to Aroclor 1254 (30mg/kg/day; 15 days). * indicates greater than control, and bars with different letters are significantly different ($p<0.05$).

The degree to which PCBs constitute a human health hazard is not yet clear and the potential ability of these substances to compromise neuroendocrine systems has not been fully investigated. To begin to address the potential deleterious effect of Aroclor 1254 on MNCs, SON punches were removed from adult male rats 4-4.5 hr after ip injection of 3.5 M saline (stimulated) or normal saline (unstimulated) and their VP release was measured. In agreement with others[2], Figure 1 shows a significant compensatory increase in VP secreted within the SON of stimulated animals, (blue bar), compared to unstimulated SON (white bar) (4.62±0.57 n=4 vs. 1.81±0.21 n=8 pg/ml/µg; $p<0.05$). SON VP is attenuated in PCB exposed rats, as SON VP release after dehydration is diminished compared to that of PCB-naïve dehydrated rats (brick red bar vs. blue bar). However, the most striking comparison revealed by Figure 1 is the apparent inability of the PCB challenged system to respond with intranuclear VP release under stimulated conditions. Whereas there is a large response to osmotic stimulation in naïve animals (blue bar vs. white bar), in the PCB exposed rats this response is virtually eliminated (brick red bar vs. gray bar).

A finding of particular interest is that *in vitro* application of Aroclor (20 uM) for 10-min (green bar) directly to SON punches from hyperosmotic rats significantly reduces the secretion of VP SON as compared to PCB-naïve stimulated animals (blue bar) (2.1±0.309 n=12 vs. 4.62±0.57 n=4 pg/ml/µg; $p<0.05$). *In vitro* application of PCBs to SON taken from PCB-naïve animals is noteworthy because this reduced preparation is functionally isolated from any possible effects of PCBs on osmoreceptors in the subfornical organ (SFO) or organum vasculosum of the lamina terminalis (OVLT), which act presynaptically upon MNCs to influence plasma VP release in the intact rat[27]. Furthermore, statistical analysis of tail blood osmolality reveals that PCBs do not alter the animal's systemic response to osmotic stimuli. These data, in combination with the inhibitory effect of *in vitro* application, suggest that the toxin is probably acting directly upon the MNCs. The deleterious effects of PCBs upon intranuclear VP may extend to systemic (plasma) output. Pilot data in Figure 2 shows that PCB-treated rats had an exaggerated increase in plasma VP of 500% over baseline in response to the saline stimulus (ANOVA on ranks; $p=0.0006$). This exaggerated disparity between normosmotic and hyperosmotic groups suggests that plasma VP output in osmotically stressed PCB-exposed rats might deplete the available pool of VP peptide more rapidly in these animals. Consistent with this speculation is the finding that in pilot studies the basal plasma levels of VP appear to be lower in PCB rats as compared to normal rats (1.0 ± 0.1 vs. 2.9 ± 0.6). Collectively, these data imply that PCBs act somewhat directly on MNCs, dampening intranuclear VP release in response to osmotic stress and eliciting relatively exaggerated plasma VP output in PCB-treated animals.

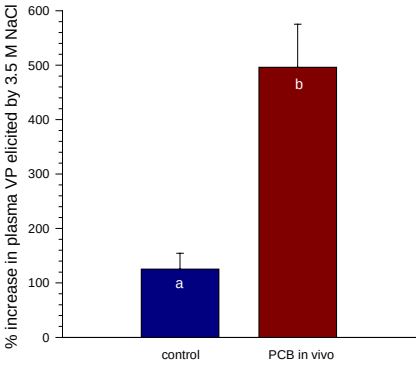
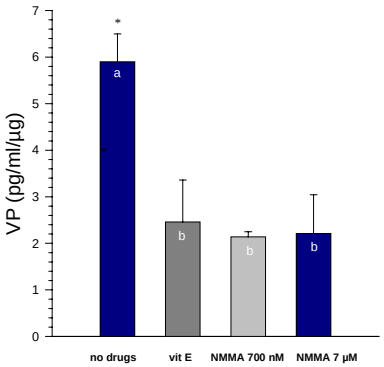


Figure 2. Percent increase in plasma VP relative to baseline in response to 3.5 M ip saline in normal and PCB-fed rats. Bars a and b are significantly different.

Figure 3. Intranuclear VP release after 3.5 M ip saline is reduced by inhibition of NO production and diffusion. * indicates sig. increase relative to normosmotic controls and bars with different letters are significantly different ($p<0.05$).



A dosage of 30/mg/kg/day for two weeks may at first be criticized as artificial and acute. However, this dose regimen was carefully chosen based on several criteria. Aroclor 1254 is a commercial mixture that has been extensively used and dispersed from industrial applications worldwide and which accumulates in high levels in food fish in the Great Lakes, Lake Tahoe and San Francisco Bay[28,29]. Moreover, the brief lifespan of our experimental model (i.e., rat) coupled with the expediency of obtaining brain levels of PCBs within the time frame of a single experiment necessitates daily exposure to PCBs at a level that might exceed expected daily human intake in most populations.

Which neurochemical processes in MNCs might account for PCB effects on the central and peripheral output of these cells? Nitric oxide (NO), a gaseous neurotransmitter synthesized from L-

arginine by NO synthase (NOS), has important functions in the regulation of body fluid homeostasis[30]. NOS expression in MNCs of the supraoptic and paraventricular nuclei is among the highest observed in the brain[32] and its activity is upregulated by dehydration in rat SON. Conversely, the NO scavenger vitamin E, and the NOS inhibitor N^G-monomethyl-L-arginine (NMMA) significantly reduce the compensatory release of VP within the SON in osmotically challenged animals[8], as shown in Figure 3. These findings suggest that during intense stimulation, NO is essential for elevated intranuclear release. Therefore, it is plausible that PCBs may disrupt the delicate regulation of intranuclear and/or systemic VP release by altering NOS activity within the SON, a hypothesis consistent with the documented inhibitory effects of PCB on brain NOS[31]. While it remains to be seen whether NO-dependent VP release is a target of PCB action, the NOS pathway appears to be convergent target of both VP itself and PCBs. In light of the preliminary data reported here, it is tempting to speculate that reduced intra-SON release of VP during dehydration may compromise the capacity for osmoregulation during prolonged physiological demand.

Acknowledgments

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