Dietary Exposure to Aroclor 1254 Alters Central and Peripheral Vasopressin Release in Response to Dehydration in the Rat

Cary G. Coburn,*1 Elizabeth R. Gillard,† and Margarita C. Currés-Collazo*†

*Environmental Toxicology Program and †Department of Cell Biology & Neuroscience, University of California at Riverside, Riverside, California 92521

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Central vasopressin (VP) release from magnocellular neuroendocrine cells (MNCs) in the supraoptic nucleus (SON) occurs from their somata and dendrites within the SON several hours after acute dehydration, and is an important autoregulatory mechanism influencing the systemic release of VP from MNC terminals in the posterior pituitary. To begin to explore the impact of polychlorinated biphenyls (PCBs) on brain mechanisms of body fluid regulation, both central and systemic VP release in response to acute dehydration were assessed in adult male rats fed the commercial PCB mixture Aroclor 1254 (30 mg/kg/day) for 15 days. Water intake and body weight were recorded daily, and on day 15 rats were dehydrated by intraperitoneal injection of 3.5 M saline (controls received physiological saline) and sacrificed 4–6 h later. Intranuclear VP release was measured in SON tissue punches in vitro, and systemic VP release was measured in the same rats. SON prepared from dehydrated PCB-naive rats released significantly more VP than did SON from control rats (4.9 vs. 2.7 pg/ml/μg). In contrast, while Aroclor 1254 exposure had no effect on baseline water intake, weight gain, or plasma osmolality responses to dehydration in PCB-fed rats, the SON failed to respond with increased VP release during dehydration. Consistent with previous studies showing an inhibitory effect of central VP on plasma VP output, dehydrated PCB-fed rats had an exaggerated 863% increase in plasma VP over basal levels, compared to a 241% increase in PCB-naïve rats, suggesting that the MNC system is subtly disrupted.

Key Words: osmoregulation; magnocellular neuroendocrine cells; polychlorinated biphenyls; somatodendritic release; supraoptic nucleus.

Despite the importance of systemic osmoregulation, the potential impact of persistent organic pollutants such as polychlorinated biphenyls (PCBs) on body fluid regulation remains virtually unexplored. Exposure to PCBs in utero has been reported to enhance intake of saline solution in rats (Hany et al., 1999), and exposure to another organochlorine, methoxychlor, increases basal water intake (Ferguson et al., 2000), raising the possibility that body fluid homeostasis might be subtly disrupted by organochlorine pollutants. Here we investigate the effects of dietary PCB exposure on hypothalamic osmoregulatory mechanisms responsible for the maintenance of optimal salt and water balance. The magnocellular portion of the hypothalamo-neurohypophysial system is a potential target of PCBs because these cells are exceptionally vascularized (Gross et al., 1986), and their axon terminals project outside of the blood brain barrier (Weiss and Cobbett, 1992), increasing the likelihood of accumulation of toxins in these cells. Magnocellular neuroendocrine cells (MNCs) of the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus respond to osmotic signals conveyed by the circumventricular organs and to the osmotic pressure of the local microenvironment by releasing oxytocin (OXY) and vasopressin (VP) into circulation from axon terminals in the neurohypophysis (Summy-Long and Kadekaro, 2001). Along with oxytocin, which promotes salt excretion (Summy-Long and Kadekaro, 2001), VP is released from MNC axon terminals with short latency following acute dehydration in vivo (Ludwig et al., 1994). Vasopressin, which is the focus of this study, acts on the kidney and on extra-renal sites to promote water conservation (Summy-Long and Kadekaro, 2001). Increased drinking and the OXY/VP-induced changes in salt and water excretion promote recovery of normal plasma osmolality (Summy-Long and Kadekaro, 2001). In addition to releasing VP systemically, MNCs also secrete VP locally from their cell bodies and dendrites within the SON. The physiological significance of this intranuclear release is not completely understood. In contrast to systemic (plasma) VP release, intranuclear release of VP is delayed by several hours, coinciding with declining peripheral VP release (Ludwig et al., 1994). Studies using direct SON infusion of hypertonic saline show that MNCs are intrinsically osmosensitive and release VP intranurally during the hypertonic stimulus (Ludwig, 1998); this VP release is even more pronounced upon a return to normosmotic solution (Ludwig, 1998). This suggests that the most effective stimulus for intranuclear VP release may be a relative decrease in the osmolality of the microenvironment (Ludwig, 1998). This is consistent with the delayed onset of intranuclear release in response to acute intravenous dehydration in vivo, when plasma osmolality is declining (Ludwig et al., 1994).
Several lines of evidence suggest that intranuclear VP may act as a short-loop feedback signal to modulate systemic VP release. Vasopressin has direct postsynaptic effects on MNCs, decreasing their electrical activity (Ludwig and Leng, 1998) or regularizing their firing patterns (Gouzenes et al., 1998) to favor those promoting efficient systemic VP release (Moos et al., 1998). In addition, OXY/VP act presynaptically to reduce excitatory input to MNCs (Kombian et al., 1997), and also reduce excitatory amino acid levels in the SON in vitro (Currás-Collazo et al., 2003). Moreover, blockade of central VP receptors exaggerates dehydration-induced systemic VP release in the intact rat (Liu et al., 1996), suggesting that intranuclear VP might act to prevent excessive VP release during increased physiological demand.

In contrast to the inhibitory influence of intranuclear VP on systemic VP output, endogenous SON VP augments its own intranuclear release in response to systemic dehydration in a nitric oxide-dependent manner (Gillard et al., 2003, 2004). Administration of nitric oxide synthase (NOS) inhibitors exaggerates systemic VP release (Kadekar et al., 1998) and increases dehydration-stimulated pituitary VP depletion (Kadowaki et al., 1994), suggesting that NO production in the SON might operate in serial and/or in parallel with intranuclear VP release to moderate systemic VP release.

Neuroactive effects of ortho-substituted PCBs and Aroclor 1254, a commercial PCB mixture containing a prevalence of such congeners, include altered neurotransmitter levels and disruption of intracellular signaling (Kodavanti and Tilson, 2000). Because Aroclor 1254 (Kang et al., 2002) and its constituent congeners (Sharma and Kodavanti, 2002) have been shown to inhibit brain NOS activity, we hypothesized that the potential effects of PCBs might include dampened VP release from MNC soma and dendrites, and/or exaggerated systemic VP release during dehydration. To test this, we measured SON and plasma VP release in both normal and dehydrated rats after dietary exposure to Aroclor 1254 (30 mg/kg/day for 15 days), similar to a regimen (30 mg/kg/day excluding weekends for 28 days) shown by others to yield brain concentrations of 8.2–15.1 ppm, or 20–50 μM (Kodavanti et al., 1998a; Kodavanti and Tilson, 2000). Such tissue concentrations have been shown to disrupt neuronal signaling without producing overt cell death (Kodavanti and Tilson, 2000).

MATERIALS AND METHODS

Animals and in vivo Aroclor 1254 dosing regimen. A total of 53 adult male Holtzmann rats (300–400 g) were used in this study. The experiment was run in four separate trials, each consisting of 8–16 subjects. Each run included the various experimental conditions, and the trends observed in the data were consistent across runs. A single lot (# 124-191-B) of Aroclor 1254 purchased from Accustandard (New Haven, CT) was used for these studies.

Rats were individually housed in a vivarium with a 12:12 h photoperiod and maintained with ad libitum access to standard rat chow pellets and water until the day of sacrifice, unless otherwise noted. Animals were weighed daily for accurate dosing (30 mg/kg/day for 15 days), and daily water intake was measured and later standardized to rat weight and expressed as ml/100 g body weight. To overcome potential neophobia to the PCB delivery vehicle, animals were subjected to a seven-day familiarization period during which cheese puffs were provided as daily supplements to their regular diet (Coburn et al., 2004). On the first day of the dosing regimen, rats were randomly assigned to either the PCB group or the control (PCB-naïve) group and each rat was provided with the appropriate amount (1 ml per kg body weight) of Aroclor 1254 (30 mg/ml suspended in corn oil vehicle) or corn oil vehicle injected into a cheese puff. The PCB-laced (or oil-containing) treat was immediately delivered and voluntarily consumed in its entirety within 3–5 min by each subject represented here, ensuring that a known amount of Aroclor 1254 was consumed daily by each rat for the duration of the dosing regimen. Thereafter, each animal received the appropriate treatment daily, until the final dose on the 15th day.

Systemic osmotic challenge and tissue harvest. On the 15th day of the study, both vehicle-treated and PCB-treated rats were injected intraperitoneally (ip; 0.6 cc/100 g body weight) with either 3.5 M NaCl (to produce acute dehydration) or 0.9% NaCl (physiological saline control, 0.15 M) and water was withheld until the animals were sacrificed 4.5–6 h later, during which time SON VP release has been shown to be elevated following a hyperosmotic ip injection (Ludwig, 1998). Removal of water after injection served to eliminate post-stimulus drinking as a potential confounding factor. Tail blood was collected and plasma osmolality was measured using a vapor pressure osmometer prior to sacrifice to confirm dehydration and in order to match the osmolality of the artificial cerebrospinal fluid (Locke’s solution) used for in vitro SON incubation to the plasma osmolality (values were rounded to the nearest 10 mOsm) of the rat for each tissue sample. After decapitation, brains were removed to cold, oxygenated (95%O₂/5%CO₂) Locke’s solution and SON was dissected bilaterally (yielding two separate unilateral SON samples per rat) from 1 mm coronal brain sections placed briefly on a chilled slide. The portion of the SON removed consisted of the anterior SON and part of the retrochiasmatic SON, taken from up to six coronal slices.

In vitro tissue preparation. After dissection, each unilateral SON sample was immediately transferred to an individual primary incubation well containing Locke’s solution (pH 7.4) and maintained in a slightly larger individual secondary plastic well in a water bath at 37°C. The primary wells were equipped with a membrane through which liquid could distribute into the secondary well during incubation, and remain there for sampling after the primary well (containing the tissue) was removed. This permitted both fast and efficient changing of incubation medium as well as complete recovery and separation of the tissue sample from the perfusate at the end of the incubation. Control samples (from 0.9% NaCl-injected rats) were incubated in normosmotic (290–300 mOsm, pH = 7.4) Locke’s composed of (in mM): NaCl (132), KCl (5), CaCl₂ (2), MgCl₂ (2), KH₂PO₄ (1.2), HEPES (10), and glucose (10), with ascorbate (35 mg/l), thiourea (15 mg/l) and bacitracin (400 mg/l) added to retard tissue degradation. Stimulated SON samples (from 3.5 M NaCl-injected rats) were incubated in Locke’s solution to which NaCl had been added to match plasma osmolality (typically 320–350 mOsm). Each well contained one unilateral SON sample, with a total volume of 500 μl Locke’s solution. Each SON was maintained with continuous oxygenation. Following a 30-min equilibration period, during which neurochemicals released as a result of the dissection might obscure tissue responses to the experimental manipulation, the equilibration solution was replaced with 500 μl of fresh Locke’s solution. Each SON was then incubated for an additional 10-min experimental period, after which the perfusate samples were collected.

A subset of samples from both normosmotic and dehydrated rats that ingested only corn oil vehicle in vivo (PCB) were treated with Aroclor 1254 (20 μM) in vitro by adding 1 μl of a concentrated Aroclor 1254 stock solution (prepared using dimethyl sulfoxide as the diluent) to 499 μl of Locke’s solution during the experimental period. To control for possible effects of the small quantity of dimethyl sulfoxide (DMSO) used as the PCB vehicle, a subset of corresponding samples were given the same volume of DMSO vehicle (final concentration of DMSO in all cases was 0.2%). The remainder of samples received only Locke’s solution during the experimental period.
At the conclusion of the experimental period, the primary well containing each SON sample was removed and an individual 150 µl aliquot of the Locke’s perfusate was collected from the secondary incubation well and immediately frozen at −20°C for subsequent VP analysis. Finally, each SON sample was collected separately in 400 µl of cold protease inhibitor cocktail (PIC) consisting of 10 nM Tris buffer (pH 8.4), 0.32 M sucrose, EDTA (5 mM), benzamidine (1 mM), aprotinin (2.3 mg/ml), phenylmethylsulfonyl fluoride (0.2 mM), leupeptin (10 mg/ml). Samples were homogenized in PIC using a sonic dismembrator and rapidly frozen for later protein determination, at which time they were thawed, agitated and centrifuged at 13,000 × g for 20 min at 4°C. Supernatant was discarded and the tissue pellets were resuspended in 10 mM Tris (pH 8.4) and assayed for protein using the bicinchoninic acid method (Pierce) so that VP values for each sample could later be standardized to control for variations in the amount of measurable VP due to the varying size of the SON punches.

**Quantification of VP.** VP content in perfusate samples was measured using an enzyme-immunoassay (arg8-vasopressin correlate enzyme-immunoassay or EIA kit, Assay Designs) with a sensitivity of 3.39 pg/ml of analyte. Perfusate samples were incubated in goat anti-rabbit IgG antibody-coated microtiter wells of an enzyme-immunoassay (arginine-vasopressin correlate enzyme-immunoassay or EIA kit, Assay Designs) with a sensitivity of 3.39 pg/ml of analyte. Samples in assay buffer were subsequently analyzed in the same manner as perfusate samples, and the results for the resuspensions were adjusted for subsequent analysis. Because the VP content in normal plasma is very low, plasma samples were first delipidated and plasma VP extracted using acetone-petroleum ether (according to the EIA manufacturer’s instructions), and were then concentrated by vacuum-evaporation and resuspension in 100 µl of EIA assay buffer. Samples in assay buffer were subsequently analyzed in the same manner as perfusate samples, and the results for the resuspensions were adjusted for the initial volume of plasma used for the delipidation and extraction (500 µl for samples from normosmotic rats; 250 µl for dehydrated rats) for expression in pg/ml of plasma.

**Statistical analysis.** Intranuclear (SON) VP, plasma osmolality and water intake averages represent pooled data from four separate experimental runs (each run consisted of 8–16 animals). Averages for plasma VP represent the three trials for which sufficient plasma was collected for analysis. For both SON and plasma VP, each rat contributed one pair of measurements (left and right SON; and two aliquots of plasma which were often different volumes and which underwent delipidation and extraction independently).

Vasopressin levels, body weight measure, osmolality measures and water intake data were analyzed for main effects of in vivo and/or in vitro treatments by one-way (or two-way where noted) ANOVA using Sigma Stat software. General linear model ANOVA was used where data met normal distribution/ equal variance assumptions; otherwise Kruskal-Wallis ANOVA on Ranks was used. Where overall significance (p < 0.05) was obtained, post hoc multiple comparisons (α < 0.05) were used to detect specific differences, with Student-Neuman-Keuls Test applied following General linear model ANOVA and Dunn’s Test applied following ANOVA on Ranks.

**RESULTS**

To begin to address the potential impact of PCBs on the magnocellular neuroendocrine system, SON tissue punches were removed from PCB-naïve (−PCB) and Aroclor 1254-fed (+PCB) rats 4.5–6 h after ip injection of physiological saline (normosmotic, representing basal conditions) or 3.5 M saline (dehydrated). The number of unilateral SON samples comprising each group are (from left to right): 14, 15, 21, and 20. Kruskal-Wallis ANOVA on ranks (p < 0.009), H = 11.6, 3 df. The asterisk indicates a significant increase in VP release in the SON of PCB-naïve rats in response to dehydration (Dunn’s test, p < 0.05) relative to the normosmotic value. Bars with different letters are also significantly different (Dunn’s test, p < 0.05); bars with the same letter are not different.

**FIG. 1.** Intranuclear vasopressin release in the SON of PCB-naïve (−PCB) rats and (+PCB) rats fed Aroclor 1254 (30 mg/kg/day for 15 days) and injected with either physiological saline (normosmotic control) or 3.5 M saline (dehydrated). The number of unilateral SON samples comprising each group are (from left to right): 14, 15, 21, and 20. Kruskal-Wallis ANOVA on ranks (p < 0.009), H = 11.6, 3 df. The asterisk indicates a significant increase in VP release in the SON of PCB-naïve rats in response to dehydration (Dunn’s test, p < 0.05) relative to the normosmotic value. Bars with different letters are also significantly different (Dunn’s test, p < 0.05); bars with the same letter are not different.
were pooled with those that received Locke’s solution with no vehicle added. As shown in Figure 2, in vitro application of Aroclor 1254 (20 μM for 10 min) to SON tissue from PCB-naïve, dehydrated rats obliterated the increase in intranuclear release normally occurring in response to acute dehydration. In vitro PCB application reduced VP release from dehydrated tissue to the level of normosmotic controls (both –PCB and +PCB in vitro, Fig. 2). Moreover, intranuclear VP release from dehydrated tissue in the presence of Aroclor 1254 was markedly and significantly lower than in the absence of PCBs (2.1 ± 0.3 compared to 4.9 ± 0.8 pg/ml/μg). Interestingly, intranuclear VP release from dehydrated tissue appears to be even lower when PCBs are applied in vitro than when PCBs are ingested (2.1 ± 0.3 compared to 2.7 ± 0.4 pg/ml/μg respectively, Figs. 1 and 2), but this effect was not significant. These findings show that the acute, direct action of Aroclor 1254 on SON tissue produces an effect similar to (though potentially more efficacious than) that produced by chronic ingestion.

It is also noteworthy that, similar to Aroclor 1254 ingestion, direct application of PCB to SON tissue did not detectably alter basal intranuclear VP release in normosomatic conditions (3.2 ± 0.5 vs. 2.7 ± 0.4 pg/ml/μg for +PCB in vitro and –PCB, respectively), as shown in Figure 2. Consistent with the lack of effect of either route of PCB exposure on the basal somatodendritic release of VP from MNCs, analysis of basal plasma osmolality on the day of sacrifice showed no detectable difference between PCB-naïve and PCB-treated rats injected with physiological saline (Fig. 3). Interestingly, the data also show that the ability of acute systemic dehydration to produce elevations in plasma osmolality was not altered in PCB-treated animals. Hypertonic injection of saline was equally effective in both groups of rats, producing an average 13% increase in plasma osmolality in PCB-naïve rats, and an average 12% increase in PCB-treated rats.

Consistent with the lack of effect on baseline plasma osmolality values is the further finding that basal water consumption did not differ between PCB-naïve and PCB-treated rats in this study. For example, measurement of daily water intake on the final day of the dosing regimen, which should best reflect the effects of cumulative exposure, was 9.3 ± 0.4 ml/100 g body weight in control animals and 9.2 ± 0.6 ml/100 g in PCB-treated rats (Fig. 4A; p > 0.8). Analysis of daily water intake on day 7 of the dosing regimen likewise revealed no significant difference between the two groups of rats (data not shown). Similarly, Figure 4B shows that comparisons of body weight gain between the first and last day of the dosing regimen failed to reveal a significant effect of PCB treatment (p = 0.1), although there is an apparent trend toward slightly less robust weight gain in

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**FIG. 2.** Acute application of Aroclor 1254 (20 μM) in vitro greatly reduces intranuclear VP release from SON punches prepared from dehydrated rats. The number of unilateral SON samples comprising each group are (from left to right): 14, 8, 15, and 12. Kruskal-Wallis ANOVA on ranks (p < 0.003), H = 14.4, 3 df. The asterisk indicates a significant increase in VP in response to dehydration (Dunn’s test, p < 0.05) relative to the corresponding normosomatic value. Bars with different letters are also significantly different (Dunn’s test, p < 0.05); bars with the same letter are not different.

**FIG. 3.** Neither basal nor dehydration-induced plasma osmolality values are altered in PCB-treated animals. The number of samples represented by each bar are (from left to right): 11, 15, 10, and 11. Kruskal-Wallis ANOVA on ranks (p < 0.00001), H = 33.7 with 3 degrees of freedom. Asterisks for both PCB-naïve and PCB-treated groups indicate a significant increase osmolality in response to dehydration (Dunn’s test, p < 0.05) relative to the corresponding normosomatic value. Bars with different letters are significantly different (Dunn’s test, p < 0.05); bars with the same letter are not different.
the Aroclor 1254-fed animals, which on average gained nearly 10 g less over the collective time frame than their PCB-naive counterparts. This represents an average 23.1% reduction in weight gain compared to PCB-naive controls.

Concomitant with its effect on intranuclear VP release, Aroclor 1254 ingestion also has a significant impact upon systemic VP release by MNCs, as shown in Figure 5. As expected (Ludwig, 1998), acute dehydration in vivo greatly increased circulating levels of VP measured in the plasma of PCB-naive rats by more than three-fold. Likewise, dehydrated PCB-treated rats also had significantly more circulating VP than did their normosmotic counterparts (Fig. 5A), demonstrating clearly that the system is capable of responding to the stimulus with a plasma VP response. Perhaps more interesting is the finding that although the absolute level of plasma VP was not different in PCB-treated and non-treated rats, the magnitude of the dehydration-elicited systemic VP increase was exaggerated in the PCB-treated group (Figs. 5A and 5B). Figure 5B shows the dehydration-induced increase in VP as a percentage of the appropriate normosmotic control group mean for both
PCB-naive and PCB-treated rats (the volume of plasma required for VP analysis combined with the potential VP response to hemorrhage prevented repeated measures in the same rat both before and after ip injection of saline). The percentage increase in response to systemic osmotic stimulation is only 241 ± 31% in normal rats, but is 863 ± 159% in PCB-treated rats (p < 0.00004). While there was an apparent trend toward reduced basal VP secretion in PCB-fed rats (as shown in Figure 5A, PCB-treated plasma release was only roughly 41% of PCB-naive basal release), this effect failed to reach statistical significance under the experimental conditions examined in this study.

DISCUSSION

The current study is the first to explore the potential impact of PCB exposure on the magnocellular neuroendocrine system responsible for hydromineral balance. To simulate the likely route of human and wildlife PCB exposure, rats were fed food treats containing PCBs. The commercial mixture Aroclor 1254 was chosen based upon extensive past use; the accumulation of PCB congeners matching this mixture in edible fish in coastal areas (San Francisco Estuary Monitoring Program Annual Reports, 1997) and lakes of the U.S. (Gerstenberger and Dellinger, 2002); and its complement of highly chlorinated ortho-substituted neuroactive congeners (Kodavanti and Tilson, 2000) that accumulate readily in brain tissue (Kodavanti et al., 1998a).

Aroclor 1254 was fed to adult male rats at 30 mg/kg/day for 15 days. While this is much higher than the expected daily PCB intake in nature, brain PCB levels are expected to be similar to those reported following a similar dosing regimen (8.2–15.1 ppm; 20–50 µM) (Kodavanti et al., 1998b; Kodavanti and Tilson, 2000). For reference, brain PCB levels of up to 29.5 ppm (w/w) have been found in wildlife exhibiting overt neurological symptoms (Gabrielsen et al., 1995). In the high-risk population of Inuit Greenlanders, adult brain levels were over 700 µg/kg lipid in brains having 8.3% mean lipid content (Dewailly et al., 1999), values that predict a brain PCB content of roughly 58 µg/kg (58 ppb). Breast-fed infants of circumpolar populations (Ayotte et al., 2003) might be at greater risk for brain accumulation of PCBs.

Central release of neurohypophysial hormones by MNCs is an important autoregulatory mechanism influencing their systemic output in response to physiological stimuli (Ludwig, 1998; Ludwig and Leng, 1998; Moos et al., 1998). The current results demonstrate that central hormone release is subtly disrupted by oral exposure to Aroclor 1254. In PCB-naive rats, systemic dehydration stimulates a delayed 82% average increase in intranuclear VP release (Fig. 1), in agreement with previous findings (Ludwig, 1998). In contrast, in the SON removed from PCB-fed rats, this response is greatly attenuated, achieving only a 33% increase, which is not significantly different from basal release in normosmotic conditions (Fig. 1). Interestingly, basal intranuclear VP release is nearly identical in PCB-naive and PCB-fed rats (Fig. 1), suggesting that the effect of PCBs is selective, with the function of the system being noticeably impaired only during conditions of physiological stimulation.

That the reduction in dehydration-elicited intranuclear peptide release is likely to reflect direct actions on cellular components within the SON is suggested by the finding that the effect of oral PCBs can be mimicked by direct application of Aroclor 1254 (20 µM) to the SON in vitro, which abolishes dehydration-elicited VP release in the SON of PCB-naive rats (Fig. 2). Dehydrated SON receiving Aroclor 1254 in vitro released slightly (though not significantly) less VP than dehydrated SON from PCB-fed rats, despite the brevity of Aroclor 1254 application. At 20 µM, the in vitro dose of Aroclor 1254 falls within the lower range of the brain levels expected to accumulate gradually during the ingestion regimen (20–50 µM; Kodavanti et al., 1998a), but should also have more immediate and direct access to cellular components within the SON, including the MNC cell bodies and dendrites.

While the efficacy of direct in vitro PCB treatment does not exclude the contribution of indirect effects to the reduction of dehydration-elicited intranuclear VP release by oral PCBs, it does show that the direct action of Aroclor 1254 on SON tissue is sufficient to reproduce this effect. The reduced SON preparation used in our in vitro experiments is functionally isolated from any possible effects of in vitro treatments on osmoreceptors in the subfornical organ (SFO) or the organum vasculosum of the lamina terminalis (OVLT), which provide afferent input signaling dehydration to the MNCs in the intact rat (Summy-Long and Kadekaro, 2001). Likewise, the effect of Aroclor 1254 in vitro on intranuclear release cannot be a product of action on any organ system, or indeed on any cellular component not contained in the tissue punch itself.

The similarity between the effects of oral and direct PCB exposure on intranuclear VP release suggests that oral PCB treatment might act directly on MNCs. Consistent with this, the reduced intranuclear VP response to dehydration in Aroclor 1254-fed rats is unlikely to be entirely due to secondary effects on other organ systems, or on compensatory drinking behavior. Changes in the efficacy of the kidney, and/or increases in basal and dehydration-elicited drinking, might reduce the ability of hypertonic saline injection to raise plasma osmolality (Summy-Long and Kadekaro, 2001), thereby reducing the stimulus for intranuclear VP release. However, increased drinking by dehydrated PCB-fed rats relative to naive rats is precluded because drinking water was withdrawn from all subjects at the time of injection. Incomplete absorption and/or distribution of the saline injection, or increased efficiency of water conservation and/or salt excretion (a function of OXY, which was not measured in these studies) following saline injection are also unlikely to
account for the reduced intranuclear VP response in PCB rats, because hypertonic injection produced identical elevations of plasma osmolality in PCB-fed and naïve rats (Fig. 3). Moreover, that PCB-fed rats were not water-loaded prior to the injection itself is shown by the finding that basal plasma osmolality was also identical in PCB-fed and PCB-naïve rats. In support of this, we found that basal daily water intake was virtually identical in both groups of rats (Fig. 4A).

That the observed effects of oral PCBs on MNC function are not attributable to nonspecific effects of overt toxicity and/or malaise is suggested by the finding that weight gain in PCB-fed rats during the study was not significantly different from that of their control counterparts. Neither is the deficit in intranuclear VP responses likely to result from overt neurotoxicity, since preliminary results from our laboratory show no cell loss within the SON in similarly treated rats (unpublished results). Collectively, the lack of effect of oral PCBs on baseline VP release, plasma osmolality, drinking and body weight gain, in combination with the efficacy of in vitro application, are consistent with direct actions on components of the hypothalamic-neurohypophysial system present within the SON.

While the exact mechanism(s) and cellular target(s) of Aroclor 1254’s effects in the current study remain unknown, Aroclor 1254 (Kang et al., 2002) and its constituent congeners (Sharma and Kodavanti, 2002) have been reported to inhibit nitric oxide synthase activity. It is noteworthy that the inhibition of nitric oxide production in SON tissue punches produces a virtually identical, selective effect on dehydration-stimulated intranuclear VP release in vitro (Gillard et al., 2004), and has been reported to exaggerate pituitary depletion of VP in the intact dehydrated rat (Kadowaki et al., 1994). While the SON contains local interneurons (Armstrong, 1995), glia (Hatton, 1990) and presynaptic elements in addition to MNCs, NADPH-diaphorase staining, a histochemical marker for NOS activity (Matsumoto et al., 1993), is elevated selectively within the MNC cell bodies of dehydrated rats (Kadowaki et al., 1994; Leon-Olea et al., 2002), suggesting that inhibition of NO synthesis in the SON might particularly target these cells.

Because intranuclear VP (Liu et al., 1996; Ludwig and Leng, 1998; Wang et al., 1982) and NO (Kadecaro et al., 1998; Kadowaki et al., 1994) production have similar inhibitory effects on systemic VP release, we hypothesized that PCB-induced inhibition of intranuclear VP and/or NO production might result in exaggerated plasma VP release in PCB-fed rats. As shown in Figures 5A and 5B, concomitant with dampened intranuclear VP release, PCB-fed animals exhibit an exaggerated plasma VP increase of over 800% in response to dehydration.

It remains to be shown whether the effects of dietary PCBs extend to the PVN as well as the SON, and whether they constitute a serious impairment of the MNC osmoregulatory system. However, the impact of PCBs could have consequences for the strict control of plasma osmolality during increased physiological demand. Exaggerated systemic VP release in response to an acute stimulus such as that presented here might cause plasma osmolality to undershoot the optimal normal value, resulting in dilution of the electrolytes important for cellular excitability (Schrier and Martin, 1998). Moreover, exaggerated plasma VP output in PCB-exposed rats during prolonged dehydration might deplete pituitary content of VP, the primary source for sustained output (Nordmann and Labouesse, 1981), more rapidly in these animals, as is the case with pharmacological inhibitors of NOS (Kadowaki et al., 1994).

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