Estrogen-Like Neurochemical and Reproductive Effects of the Major Metabolite of 3,4,3',4'-Tetrachlorobiphenyl (3,5,3',4'-Tetrachloro-4-biphenylol)

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Abstract

We have demonstrated that intraperitoneal injection of the coplanar polychlorinated biphenyl (PCB) congener 3,4,3',4'-tetrachlorobiphenyl (TCB) significantly elevates uterine wet weight in the prepubertal rat. In addition, following *in-vivo* microdialysis of 3,5,3',4'-tetrachloro-4-biphenylol, the major metabolite of 3,4,3',4'-TCB, into the striatum of awake adult ovariectomized animals, we report significant elevations in striatal dialysate concentrations of L-DOPA and dopamine. These results suggest that the previously reported elevations in brain dopamine concentrations seen in rats perinatally exposed to 3,4,3',4'-TCB may be due to the estrogen-like activity of its metabolite 3,5,3',4'-tetrachloro-4-biphenylol.

Introduction

We have recently demonstrated [1] that developmental exposure (*i.e.*, gestational day 6 through weaning) to the coplanar congener, 3,4,3',4'-TCB, significantly elevates dopamine (DA) concentrations in the frontal cortex and substantia nigra of offspring sacrificed at postnatal days (PND) 35, 60 and 90. These results were unexpected since exposure of cells in culture [2,3] and adult animals [4,5] to either individual PCB congeners or Aroclor mixtures results in decreases in cellular and brain concentrations of DA. Furthermore, the relative activity of this coplanar congener (dams were exposed to either 0.1 or 1.0 mg/(kg·day)) brings into question the structure activity relationships that have demonstrated, using either pheochromocytoma (PC12) cells [3] or cerebellar granule cells [6], that only non-coplanar PCB congeners are active.

Considerable experimental evidence has demonstrated that 17-ß estradiol (E_2), administered either exogenously or elevated during the normal estrus cycle, increases DA turnover and elevates brain DA concentrations [7,8]. We have thus hypothesized that the elevations in brain DA seen following perinatal exposure to 3,4,3',4'-TCB may be due, in part, to the estrogen-like activity of either the parent congener or its hydroxylated metabolite. In order to test the above hypothesis we have determined: (i) the estrogen-like activity of 3,4,3',4'-TCB by measuring uterine weight in prepubertal animals and (ii) the neurochemical response to either E_2 or 3,5,3',4'-tetrachloro-4-biphenylol administered into the striatum of adult ovariectomized adult rats by *in-vivo* microdialysis.

Experimental Methods

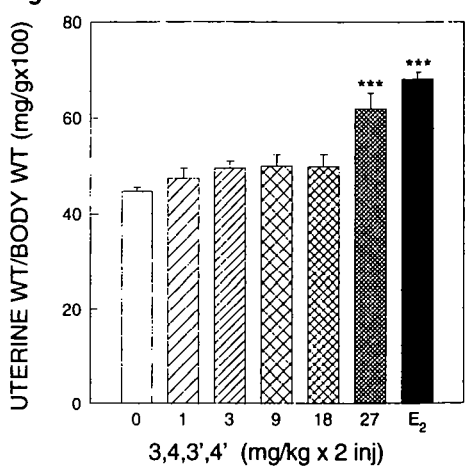
Prepubertal Sprague-Dawley derived rats obtained from the breeding facility of the New York State Department of Health were injected, on PND 21 and 22 with two injections of corn oil or varying concentrations of 3,4,3',4'-TCB, or corn oil on D21 followed by a single injection of 20 ng/kg of E_2. Animals were sacrificed 24 hrs after the second injection and the uteri were removed, trimmed of connective tissue and weighed. All dissections were conducted blind. Results are expressed as uterine wet weight/body weight ratios.

For the *in-vivo* microdialysis experiments adult ovariectomized Sprague-Dawley derived rats had guide cannula stereotactically implanted in the striatum and allowed to recover from surgery for a minimum of four days prior to testing. On the evening prior to collection of neurochemical data the obturator was removed from the guide cannula and a Carnegie-Medicin microdialysis probe was carefully placed in the guide cannula. Artificial cerebrospinal fluid (CSF) was pumped at a rate of 1 µl per minute. The next morning thirty minute collections of dialysate were collected for analysis of L-DOPA, DA and DOPAC by high-performance liquid chromatography [9]. Following a two hr baseline collection, E_2, 17-α estradiol (each at 2 nM in CSF) or 3,5,3',4'-tetrachloro-4-biphenylol (20 and 200 nanomolar in CSF) were administered for two hours. Following this exposure period, the animals were dialyzed for an additional two hours with CSF to determine if the neurochemical indices returned to baseline values. Neurochemical data from the thirty-minute collection intervals were averaged for the baseline, exposure and recovery periods for statistical and graphical purposes. Data for all experiments were analyzed using 1-way analyses of variance combined with post-hoc Boniferonni-corrected t-tests.

Results and Discussion

Intraperitoneal exposure of prepubertal rats to the coplanar congener 3,4,3',4'-TCB resulted in significant increases in uterine/body weight ratios following exposure at 27 mg/kg (Fig. 1). These results support the earlier findings that this coplanar PCB congener increases the number of foci in MCF-7 cells (Gierthy, work in preparation) and strongly suggests that either the parent congener or a metabolite possesses estrogen-like activity.

Figure 1



In-vivo microdialysis of 2 nM E_2 significantly elevated L-DOPA and DA concentrations in dialysate collected from the striatum of adult ovariectomized rats. However, microdialysis of 17-α estradiol did not alter DA neurochemistry (Figs. 2A and 2B) demonstrating that the elevations in L-DOPA and DA were **not** due to non-specific perturbations of the neuronal membrane and instead reflect the actions of E_2 on DA synthesis. More importantly, microdialysis of the metabolite of the coplanar PCB congener 3,4,3',4'-TCB (at concentrations in CSF of 20 and 200 nM) also resulted in significant elevations in dialysate concentrations of L-DOPA and DA (Figs. 3A and 3B). Except for DA concentrations in the 200 nM 3,5,3',4'-tetrachloro-4-biphenylol exposed animals, all neurochemical values returned to baseline levels during the recovery period. The similarity of the time course and magnitude of the elevations strongly suggest that this metabolite may be estrogenic.

Figure 2

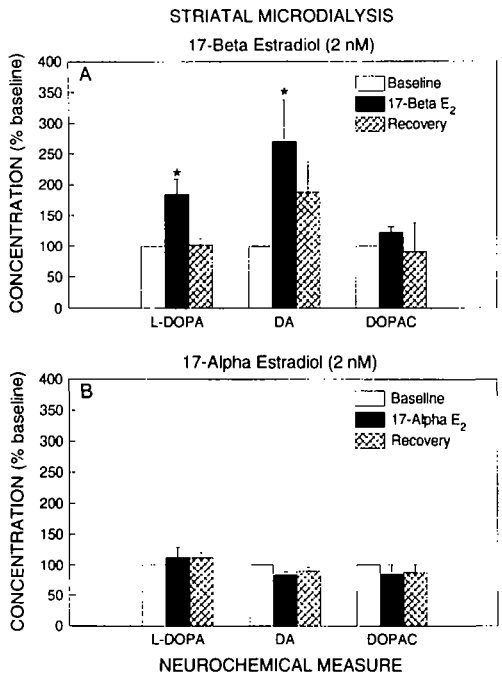
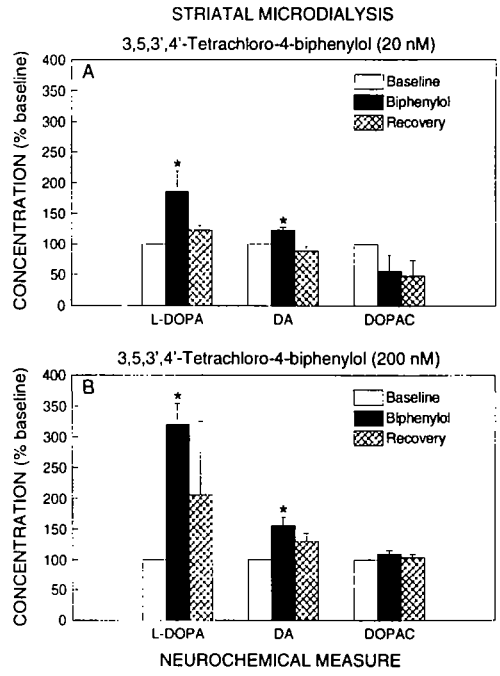


Figure 3



We are now, perhaps, in a better position to explain the elevations in brain DA concentrations that we have reported following perinatal exposure of the rat to the coplanar PCB congener 3,4,3',4'-TCB. Following a similar exposure paradigm, Morse *et al.* [10] reported significant accumulation of the hydroxy biphenylol metabolite of 3,4,3',4'-TCB (*i.e.*, 3,5,3',4'-tetrachloro-4-biphenylol) in the brains of fetuses and weanling animals. Results from the prepubertal uterine weight assays (and the neurochemical *in-vivo* microdialysis experiments) demonstrate that either the parent congener, or more likely, its hydroxy metabolite is estrogenic. Furthermore, the microdialysis experiments clearly demonstrate that both E_2 and the hydroxy metabolite of 3,4,3',4'-TCB elevate L-DOPA and DA dialysate concentrations. This latter finding strongly suggests that the elevations in brain DA following developmental exposure to 3,4,3',4'-TCB, the increases in uterine weight wet following acute exposure to the coplanar congener and the increased dialysate concentrations of L-DOPA and DA are due to the estrogen-like actions of 3,5,3',4'-tetrachloro-4-biphenylol.

Although we have conducted the *in-vivo* microdialysis experiments in the striatum, we would expect to see similar elevations in DA function in other brain areas that are more closely linked with the kinds of behavioral dysfunctions that have been observed following developmental exposure to individual PCB congeners. Indeed, following perinatal exposure of the rat to 3,4,3',4'-TCB we observed highly significant elevations in frontal cortical DA. In turn, both Murphy *et al.* [11] and Verma and Moghaddam [12] have reported that elevations in frontal cortical DA in both rats and monkeys induced by pharmacologic means result in significant deficits on cognitive tasks including spatial alternation. Murphy *et al.* [11] conclude that there may be a critical range of dopaminergic activity for optimal frontal cortical-dependent cognitive functioning and that exceeding this range may result in dysregulation and cognitive impairment.

In summary, these experiments demonstrate that the hydroxy metabolite of 3,4,3',4'-TCB is estrogen-like and is capable of significantly elevating both L-DOPA and DA dialysate concentrations. The elevations in DA function following developmental exposure to an environmentally relevant coplanar PCB congener [1] is of sufficient magnitude to adversely affect performance on measures of cognitive function often used following exposure of rodents to PCBs.

Acknowledgements

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