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Authors

Agrawal, AK

Tilson, HA

Bondy, SC

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3,4,3',4'-TETRACHLOROBIPHENYL GIVEN TO MICE PRENATALLY PRODUCES LONG-TERM DECREASES IN STRIATAL DOPAMINE AND RECEPTOR BINDING SITES IN THE CAUDATE NUCLEUS

A.K. AGRAWAL, H.A. TILSON and S.C. BONDY

Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709 (U.S.A.)

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SUMMARY

Pregnant CD-1 mice were given 32 mg/kg of 3,4,3',4'-tetrachlorobiphenyl (TCB) or corn oil vehicle, by gavage, on days 10-16 of gestation. At 1 year of age, the offspring were tested for spontaneous motor activity; the mice were then killed and dopamine (DA) levels and specific DA receptor binding were measured in the corpus striatum. Mice exposed to TCB in utero had elevated levels of motor activity, which were associated with decreased DA levels and DA receptor binding sites. The results indicate that in utero exposure to TCB might permanently alter the development of striatal synapses.

INTRODUCTION

Mice exposed to TCB during gestation have been reported to exhibit a long-lasting neurobehavioral syndrome consisting of stereotypic head movements, rotational behavior, increased motor activity, impaired neuromuscular strength and coordination, and learning deficits [1]. Other investigators [2, 3] have also noted that in utero exposure to TCB produces hyperactivity beginning at about 2 weeks of age and lasting up to at least 8 months of age.

Chou et al. [3] examined mice exhibiting the TCB-induced neurological syndrome and found alterations in the synapses of the nucleus accumbens of adult mice

Abbreviations: ANOVA, analysis of variance; DA, dopamine; LSD, least significant difference; PCB, polychlorinated biphenyl; TCB, 3,4,3',4'-tetrachlorobiphenyl.

exposed in utero to TCB; these observations suggest that TCB might have interfered with the synaptogenesis of dopaminergic systems. Possible alterations in dopaminergic function were also suggested by the observation that haloperidol, a DA receptor antagonist, decreased the overall motor activity of mice exposed to TCB more than controls.

That TCB might produce persistent alterations in DA receptor function correlated with a neurological syndrome consisting of hyperactivity prompted us to explore the long-term effect of TCB on the levels of DA and specific dopamine receptor binding in the corpus striatum, an area believed to be involved in the control of stereotypic motor movements. Mice exposed to TCB during gestation and showing signs of spinning at about 14 days of age were compared to non-spinning littermates also exposed in utero to TCB and to control animals whose mothers received corn oil vehicle. The longest period at which TCB-exposed mice have been assessed is 8 months following birth [3]. The present experiment extended this period of observation to 1 year postnatally in order to assess the irreversible nature of the neurological syndrome.

METHODS

Subjects and prenatal exposure

Pregnant female CD-1 mice (Charles River, Wilmington, MA) were randomly assigned to test groups, caged individually and provided with free food and water. The animals were treated orally on days 10–16 of gestation (morning of sperm plug was designated as day 0 of pregnancy) with 32 mg/kg of TCB or with 0.04 ml of corn oil vehicle/g of body weight. Gas chromatographic/mass spectroscopic analysis of the TCB (synthesized at NIEHS, Research Triangle Park, NC) indicated a purity of 97.7%.

The day after birth, the young were counted and examined for gross abnormalities. At 4 and 21 days of age, they were sexed, weighed, and examined for the neurological syndrome produced by TCB. Mice displaying repeated head bobbing and rotational movements during a 5–10-min observational period were defined as 'spinners'. Littermates not exhibiting these behavioral characteristics were defined as 'non-spinners'.

The pups were housed with their natural mothers up to 21 days of age, after which they were rehoused according to exposure (corn oil or TCB), syndrome (spinner or non-spinner) and sex. Animals were housed in groups of 4–6 in plastic cages kept in air-conditioned quarters having a relatively constant temperature ($21 \pm 2^\circ\text{C}$) and humidity ($50 \pm 10\%$) and 12 h light/dark cycle (light, 7 a.m. to 7 p.m.). At 1 year of age, 8 spinners, 8 littermates of the spinners which did not develop the neurological syndrome and 8 age-matched controls were tested.

Body weights and activity measures

At one year of age, the mice were weighed and then tested for horizontally directed spontaneous motor activity. Animals were placed individually into small plastic cages having the same dimensions as their home cage but not containing bedding and placed onto a commercially available activity monitor (Automex, Columbus Instruments; Columbus, OH) contained inside a sound-attenuated outer chamber equipped with a ventilation fan. Activity was measured in the darkened cubicles for a period of 30 min. All testing was done between 10.00 and 14.00 h.

Neurochemical analyses

Approx. 2 weeks after being behaviorally tested, the mice were killed by decapitation and the brain quickly removed. The corpus striatum was dissected [4] in an ice bath and frozen at -20°C until analysis.

DA concentrations and specific receptor binding in the striatum were determined from the same tissue by homogenizing the striata in 3 ml of 0.1 M phosphate buffer and centrifuging at $50\,000 \times g$ at ca. 20 000 rev./min in a Sorvall RC2B centrifuge for 10 min. The supernatant was saved for measurement of DA levels using the fluorimetric method of Jacobwitz and Richardson [5]. DA levels were determined by use of a series of standards. Sample readings were corrected for blank values and counting efficiency by dividing the obtained value by the mg of protein per tissue to obtain μg of DA/g protein. The pellets were saved for DA receptor binding assay. Crude membrane was prepared by resuspending the pellet in water and centrifuging again at $50\,000 \times g$ for 10 min. The pellets were finally suspended in 40 mM Tris-HCl buffer (pH 7.4) resulting in a concentration equivalent to 50 mg of original tissue/ml.

Receptor binding measurements were carried out in a final volume of 1 ml containing 40 mM Tris-HCl pH 7.4, 10^{-9} M 1-phenyl-4-[^3H]spiroperidol (25.6 Ci/mmol, New England Nuclear Corp.) and an amount of tissue corresponding to 5 mg original wet weight (350–400 μg protein). Protein concentration was determined by the method of Lowry et al. [6]. Incubation was at 37°C for 10 min. To determine the level of non-specific binding, half of the incubations were carried out in the presence of 10^{-6} M haloperidol. At the end of incubation, samples were filtered on glass fiber filters (20 mm diameter, 0.3 μm pore size, Gelman Inc. Ann Arbor) and washed 3 times rapidly with 5 ml Tris buffer. Filters were then dried and counted in 5 ml of a scintillation mixture (Aquasol, New England Nuclear Corp., Boston, MA) at an efficiency of 38–43%. Specific DA binding ([^3H]spiroperidol) was likewise corrected for background and divided by the protein present to obtain pmol bound/g protein.

Previous studies with rats have indicated that the binding reaction reaches equilibrium when carried out under these conditions and is reversible,

stereospecific, and proportional to the amount of membrane in the reaction mixture [7]. Non-specific binding is always less than 20% of total binding.

Statistical analyses

Overall treatment effects on body weight, DA levels and DA binding were tested for statistical significance using a one-way ANOVA [8]. Motor activity measures were expressed as rates per min, square-root transformed [9] and analyzed by ANOVA. After significant overall effects were observed, differences between individual groups were tested for significance using Fisher's LSD Test [10].

RESULTS

General health and body weights

At 1 year of age, the average (\pm S.E.) weight of the control animals was 35.8 ± 1.6 g, while the spinner and non-spinners weighed 30.0 ± 1.6 g and 30.6 ± 1.6 g, respectively. ANOVA of the body weight data showed that there was a significant overall effect, $F(2,21) = 3.89$; $P < 0.0364$; pairwise comparisons between groups showed that the controls weighed significantly more than the TCB-exposed mice. The TCB spinners and non-spinners did not differ statistically. With the exception that the TCB non-spinners had lower body weights than controls, these observations are consistent with those of Chou et al. [13].

Motor activity

ANOVA of the activity measures indicated a significant treatment effect [$F(2,21) = 3.47$; $P < 0.0500$]. Pairwise comparisons of the groups revealed that the TCB spinners were significantly more active than the controls (Fig. 1). The TCB non-spinners had higher activity scores than the controls, but the difference was not statistically significant. The TCB non-spinners also did not differ statistically from the TCB spinners.

Effects on the striatal dopamine system

ANOVA indicated that there was a significant treatment effect on DA levels in the corpus striatum, $F(2,21) = 9.15$; $P < 0.0015$. Pairwise comparisons between groups showed that the DA levels of the TCB spinners were significantly less than those of the controls (Fig. 2A). The TCB non-spinners appeared to have lower DA levels than controls, but the effect was not statistically significant. The DA levels of the TCB non-spinners were significantly higher than the TCB spinners.

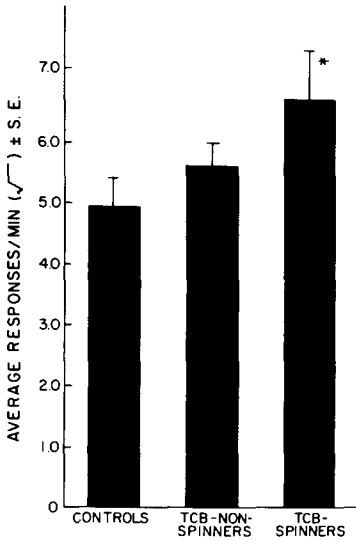


Fig. 1. The effects of in utero exposure to TCB on motor activity at 1 year of age. Mice were placed individually into activity monitors and activity was counted for 30 min. Data are square root transformations of average rate (cpm) for 8 mice per group. Data were analyzed for overall significance using a one-way ANOVA; asterisk indicates a statistical difference from control (Fisher's LSD test, $P < 0.05$).

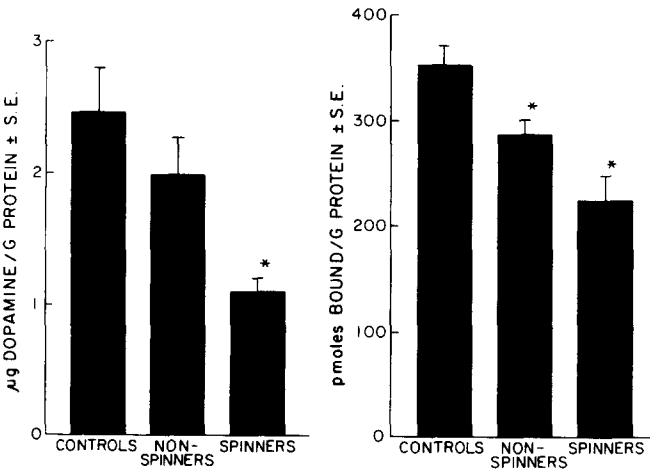


Fig. 2. The effects of in utero exposure to TCB on dopamine levels (A) and dopamine receptor binding (B) in the corpus striatum. Mice were sacrificed at 1 year of age. Data are average concentrations \pm S.E. for 8 mice per group. ANOVA was used to analyze for overall statistical significance. Asterisks indicate a statistical difference from control (Fisher's LSD test, $P < 0.05$).

ANOVA of the DA receptor binding data also indicated a significant treatment effect [$F(2,21) = 14.70$; $P < 0.0001$]. Pairwise comparisons between groups showed that the specific binding for [^3H]spiroperidol was significantly decreased in the corpus striatum of both TCB non-spinners and TCB spinners (Fig. 2B). TCB spinners had significantly less binding than the TCB non-spinners.

The neurochemical data indicate that in utero exposure to TCB can produce significant alterations in the levels and specific binding sites of DA in the corpus striatum of mice. Significant alterations in DA binding were also observed in mice not exhibiting the spinning syndrome.

DISCUSSION

The results of these experiments indicate that in utero exposure to TCB, an isomer present in some PCB mixtures, may produce a significant hyperactivity in exposed animals for up to 1 year after birth. TCB is one of the PCB isomers that is metabolized and excreted rapidly [2] suggesting that the long-term behavioral alterations of TCB might be related to a permanent structural alteration produced by the perinatal presence of the chemical. Since the nigrostriatal dopaminergic system has been implicated in the control or modulation of some types of motor movement, our data showing that TCB produced significant decreases in the levels of DA and in DA binding suggests that at least part of the long-term neurobehavioral effects of TCB might be related to the effects of the chemical on the development of the dopaminergic system. The apparent correlation between the intensity of the behavioral alterations evoked by TCB and the extent of changes in the striatal dopaminergic system further support the concept that these behavioral changes are consequent to an insult to dopaminergic circuitry. Chou and his co-workers [3] have proposed that developmental exposure to TCB might interfere with the synaptogenesis of DA neurons and might, in part, account for the spinning and hyperactivity of the TCB-exposed animals. The specificity of such toxic effects on neural circuitry involving a single neurotransmitter remains to be determined.

The hyperactivity exhibited by the TCB-spinning mice consisted of repetitive bobbing of the head and rapid circular movement. Once initiated, the spinning typically was unidirectional; no one direction (left or right) appeared to predominate, however. The stereotypic movement and unilateral spinning movements of the TCB-exposed mice support the interpretation that TCB neurological syndrome might, in part, be mediated by TCB effects on DA ontogeny. Stereotypic behavior and circling have been linked to DA function by numerous investigators [11].

Hyperactivity, as detected by relatively gross behavioral analysis, was associated with large decreases in dopamine levels and receptor binding in the TCB spinners. The TCB non-spinners, which were littermates of the TCB spinners, by definition did not exhibit the spinning syndrome, yet animals in this group tended to be more

active than controls and tended to have lower DA levels in the corpus striatum; significant decreases in DA receptor binding were observed in the TCB non-spinners. Animals exposed perinatally to TCB may not demonstrate obvious neurological deficits such as spinning, but may, in part, have very subtle neurobehavioral and neurochemical deficits lying at the edge of detectability. In an earlier study in our laboratory [1], we reported that TCB non-spinners, had fewer neurological deficits than the TCB spinners, but were deficient on more subtle tests of neurobehavioral functioning, e.g., the ability to learn a one-way avoidance task. More precise functional tests (behavioral and neurochemical) might detect more subtle developmental neurotoxicological effects of lower doses of this chemical.

The simultaneous depression of both DA levels and the extent of spiroperidol binding suggest that the normal ontogenesis of DA neurons was impaired. This might account for the excess activity of treated animals. Blockade of the DA system in adult animals, by haloperidol for example, can result in an elevation of DA binding capacity [12]. However, exposure to haloperidol during gestation can depress DA receptor density [13]. Thus, damage to the DA system during early synaptic development may have effects on receptor number that are opposite to those caused by impairment of the adult DA neurons. However, in both cases, a hyperactive state could be caused by inadequate nigrostriatal inhibition of cholinergic activity in the striatum [14].

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