Developmental Exposure to Aroclor 1254 Produces Low-Frequency Alterations in Adult Rat Brainstem Auditory Evoked Responses

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Developmental exposure of Long-Evans rats to 0, 1, 4, or 8 mg/kg/day Aroclor 1254 (A1254) from Gestational Day 6 through Postnatal Day 21 produces an elevated behavioral threshold for a 1-kHz tone. Brainstem auditory evoked responses (BAERs) were assessed in a subset of these animals (about 1 year old) using filtered clicks at 1 (65 and 80 dB SPL), 4 (60 and 80 dB SPL), 16 (40 and 80 dB SPL), and 32 (40 and 80 dB SPL) kHz. Aroclor 1254 decreased BAER amplitudes at 1 and 4 kHz, but not at 16 or 32 kHz. A dose-related decrease in the baseline-to-peak P_{1α} amplitude was observed for the 1-kHz (80-dB) stimulus. Doses of 1, 4, or 8 mg/kg/day A1254 decreased the peak-to-peak amplitude of both P_{1α}N_{1} and P_{2α}N_{2} for a 1-kHz (80-dB) stimulus. Doses of 4 and 8 mg/kg/day A1254 decreased the peak-to-peak amplitude of N_{1}P_{1} and P_{2α}N_{2} for a 4-kHz (60-dB) or 1-kHz (80-dB) stimulus. At 8 mg/kg/day, A1254 also increased the latency of peak P_{4} at 1 kHz (65 dB). The decreases in peak P_{1α} amplitudes are consistent with a dysfunction of the cochlea and/or auditory nerve. Together, the data confirm that developmental exposure of rats to A1254 produces a permanent low- to mid-frequency auditory dysfunction and suggest a cochlear and/or auditory nerve site of action.© 1996 Society of Toxicology

Widespread use, improper disposal, and environmental persistence have made polychlorinated biphenyls (PCBs) among the most prevalent environmental contaminants (Safe, 1994). The high lipophilicity and long half-lives of PCBs lead to characteristic bioaccumulation and biomagnification in both wildlife and humans (Evans et al., 1991; Hansen, 1987; Safe, 1994). Epidemiological studies of infants and children accidentally exposed to high levels of PCBs have shown a variety of neurological abnormalities, including altered activity levels (Chen and Hsu, 1994; Jacobson et al., 1990) and cognitive deficits (Chen and Hsu, 1994; Chen et al., 1992; Ko et al., 1994; Lai et al., 1994; Rogan et al., 1988). Based on reviews of both animal testing and human epidemiology studies, it is now generally accepted that PCBs are developmental neurotoxicants (Seegal and Schantz, 1994; Tilson et al., 1990).

Recent evidence has demonstrated that perinatal exposure to Aroclor 1254 (A1254) leads to a selective low-frequency hearing loss in rats (Goldey et al., 1995b). This auditory dysfunction may be secondary to the hypothyroxinemia induced during development (Collins and Capen, 1980; Goldey et al., 1995b; Morse et al., 1993; Ness et al., 1993), and may be exacerbated by the vulnerability of the developing rat auditory system to postnatal hypothyroidism (Deol, 1973; Goldey et al., 1995c; Uziel, 1986; Uziel et al., 1980, 1981). Furthermore, preliminary evidence suggests that the auditory deficit induced by PCB exposure can be attenuated by supplemental thyroxine during the preweaning postnatal period (Goldey et al., 1995a).

To date, the mechanism and site for the PCB-induced auditory dysfunction are unknown. Previous results were obtained using a behavioral procedure, reflex modification audiometry (Goldey et al., 1995b), that can detect ototoxicity (Crofton et al., 1990, 1994a,b; Fechter and Carlisle, 1990; Young and Fechter, 1983), but does not provide information as to the locus of damage in the auditory pathway. In the present study, the auditory function of animals exposed developmentally to PCBs was tested using brainstem auditory evoked responses (BAERs). BAERs were used to confirm the selective low-frequency effect previously reported and to examine possible loci of damage in brainstem auditory pathways (Moller and Jannetta, 1985; Picton, 1986; Shaw, 1988; Stockard et al., 1990).
METHODS

Animals and treatment. Subjects were a subset of the male rats dosed with A1254 and tested previously for auditory thresholds using reflex modification audiometry (Goldey et al., 1995b). Details of the housing and dosing procedures have been previously described (Goldey et al., 1995b). Briefly, primiparous Long–Evans rats were obtained from Charles River Laboratories (Raleigh, NC) on Gestation Day (GD) 2. The dams were administered A1254 (AccuStandard, Inc., New Haven, CT; CAS No. 11097-61-1; Lot No. 6024) via daily oral gavage from GD 6 through Postnatal Day (PND) 21 (except for PND 1) at doses of 0 (corn oil vehicle), 1, 4, or 8 mg/kg A1254 (dose volume of 1 ml/kg). After weaning, offspring were housed in same-sex groups of 2 (12-hr light–dark cycle, lights on 06:00; 22 ± 2°C; 40 ± 20% relative humidity) with Purina Lab Chow and tap water provided ad libitum. Animals were housed in an AAALAC-approved facility, and all investigations were approved by the National Health and Environmental Effects Research Laboratory Animal Care and Use Committee of USEPA.

Test methods. At about 350 days of age, the animals were transferred to single housing and 1 week later were implanted with electrodes for electrophysiological testing. The number of animals (all from different litters) in each dose group was as follows: 0 (n = 7), 1 (n = 11), 4 (n = 10), and 8 (n = 6) mg/kg/day. Subjects were anesthetized with sodium pentobarbital (50 mg/kg, ip) and concurrently atropinized (0.2 mg/kg, sc) to decrease respiratory distress. Epidural stainless-steel screw electrodes were implanted, using blunt ear bars, as previously described (Herr et al., 1994). The active electrode was located 3 mm posterior to lambda and 2 mm to the right of bregma. A ground electrode was located 2 mm anterior to and 2 mm to the left of bregma. Subjects were allowed 7 days of recovery before electrophysiological testing.

For recording BAERs, each unanesthetized animal was restrained in a decapine (Brainстве Scientific, Inc., Brainстве, MA), its head and pinnae exposed, and placed in a custom-designed testing apparatus contained inside a sound-attenuated Faraday box. A temperature probe (Model RET-1; Physitemp Instruments, Inc., Clifton, NJ) was inserted approximately 8 cm rectally and connected using a shielded and grounded wire to a thermometer (Model TH-8, Physitemp Instruments, Inc.) located outside the Faraday cage. Deep colonic temperature was recorded following each BAER waveform. The animals were allowed to acclimate to the test apparatus for approximately 2 min before stimulation.

Auditory stimuli were simulated filtered clicks centered at 1, 4, 16, or 32 kHz. Each frequency was presented at two intensities (peak dB SPL: re: 20 μPa; 65 and 80 for 1 kHz, 60 and 80 for 4 kHz, 40 and 80 for 16 and 32 kHz. These lower stimulus intensities were chosen such that easily recognizable waveforms (above threshold) would be recorded from control animals and 1 kHz (4 kHz) or 40 dB SPL lower than the high-intensity stimulus (80 dB SPL; 16 and 32 kHz). The stimuli were generated by a personal computer from files created using software that simulated an eighth-order Butterworth bandpass filter (PSpace, MicroSim Corp., Irvine, CA). The analog waveform output was at a minimum of 1 MHz with 12-bit accuracy using a WSB-100 Waveform Synthesizer with a WSB-A12M Waveform Synthesizer Module (Quatech, Akron, OH). The signal duration was approximately 10.1 msec. The stimulus waveform was attenuated (Model 350D, Hewlett Packard, Palo Alto, CA), amplified (Model 450, Belles Research Corp., Rochester, NY), and delivered through a leaf tweeter (Model EAS-IOTH400A, Matsushita Electronic Components Co., Ltd, Japan) placed approximately 7.7 cm in front of and 26 cm above the animal’s auditory canals. This resulted in a distance of about 27 cm between the speaker and the auditory canals, at an angle of approximately 73°. The stimulation rate was 5.56 Hz. Presentation of the different stimulus frequencies and intensities was counterbalanced over the different dose groups.

The evoked responses were amplified 10,000x and bandpass filtered (0.1–20,000 Hz; half-amplitude points; rolloff = 6 dB/octave; Model 12A5 Neurodata Acquisition System, Grass Instruments, Astro-Med, Inc., West Warwick, RI). The signals were digitized at 81,967 Hz with 12-bit resolution, using a VAX 4000-100 and ADQ32 analog-to-digital conversion boards (Digital Equipment Corp., Woburn, MA). The signals were sampled for a total of 16.5 msec, the first 1.5 msec of which served as a prestimulus baseline. One thousand waveforms were averaged for each stimulus. Peak amplitudes and latencies were measured from each animal’s average waveform. Peak amplitudes (in μV) were measured as peak-to-peak values. Additionally, the amplitudes of peaks P1 and P2 were also measured from baseline, which was defined as the average voltage over the prestimulus period. Peak latency (in msec) was calculated from stimulus onset. Peak latencies were not adjusted for the acoustic travel time (approximately 810 μsec). The interpeak latency between peaks P2 and P4 was also calculated (Picton, 1986; Sohmer et al., 1991).

The amplifier’s and computer’s amplitude and latency response factors were calibrated using sine waves of 178 μV RMS at 3 Hz, 100 Hz, and 3 kHz. Calibration of auditory stimuli was performed at ear level in the test chamber using a Bruel & Kjær Measuring Amplifier (Model 2636) with a 0.635-cm microphone (Model 4135, Bruel & Kjær, Marlborough, MA) and a 200-Hz high-pass filter (Model 3343, Krohn-Hite, Avon, MA).

Statistics. All peaks were subjected to a signal-to-noise ratio (SNR) analysis that determined the largest peak-to-peak voltage excursion occurring during the prestimulus baseline period. Only peaks that were greater than twice the noise level in control waveforms were analyzed. Data were analyzed using a repeated-measures analysis of variance (ANOVA. PROC GLM) (SAS Institute, Inc., 1989) using a Greenhouse–Geisser correction factor (Greenhouse and Geisser, 1958; Greenhouse and Geisser, 1959; Keselman and Rogan, 1980). The dose of A1254 was between-subject factor, and stimulation frequency and intensity were within-subject factors. The data for each frequency were analyzed separately because the different intensities were used for the various stimuli precluded a balanced factorial analysis. Significant main effects of treatment, or a treatment × intensity interaction, were followed by step-down ANOVAs as which examined treatment effects at each stimulus intensity. A critical α < 0.05 was used in all statistical evaluations. Group mean comparisons were performed using a Tukey–Kramer multiple comparison test (α = 0.05) (Kramer, 1956). Only data in which there were significant differences from controls are reported. Group-averaged BAER waveforms were calculated from individual animal data, and are presented for illustrative purposes.

RESULTS

Developmental exposure to A1254 decreased the amplitude of BAERs and increased some peak latencies. Decreases in peak amplitudes occurred at 1 and 4 kHz (Figs. 1, 2, 5, 6), but not at 16 or 32 kHz (Figs. 3–6). The increase in peak latency was not as dramatic as the decrease in peak amplitudes. Evoked responses were observable at all frequencies and intensities, but at 1 and 4 kHz several BAER peaks were not quantifiable due to an inadequate SNR. Additionally, peak P1b was not quantifiable at the lower intensity at any stimulus frequency (see Figs. 1–4). The BAERs were under stimulus control, as indicated by the increased amplitudes at 80 dB SPL compared with the lower stimulation intensities (Figs. 1–4). There were no significant differences in body weight either at surgery [F(3, 30) = 0.59, p = 0.6259] or at BAER testing [F(3, 30) = 0.69, p = 0.5627] (data not shown).
amplitudes of peaks $P_{1A}N_1$, $P_{1B}N_1$, $N_1P_2$, and $P_2N_2$ [$F$'s (3, 30) $\geq 5.92$, $p$'s $\leq 0.0027$]. The amplitudes of peaks $P_{1A}N_1$ and $P_{1B}N_1$ were decreased by all doses of A1254 compared with controls. The amplitude of peak $N_1P_2$ was decreased by 4 mg/kg/day A1254 compared with controls, and by 8 mg/kg/day A1254 compared with the 0 and 1 mg/kg/day doses. Peak $P_2N_2$ amplitude was decreased by 4 and 8 mg/kg/day A1254 compared with controls. No significant differences from controls were indicated for the peak-to-peak amplitude of peaks $N_2P_3$, $P_3N_3$, or $N_3P_4$ or the baseline-to-peak amplitude of peak $P_4$ at either 65 or 80 dB SPL (data not shown).

When the 4-kHz stimulus was used, the decreases in BAER amplitudes produced by developmental exposure to A1254 were more pronounced at 60 than at 80 dB SPL (Figs. 2, 5, 6). Significant main effects of treatment, or a treatment $\times$ intensity interaction, were observed for the peak-to-peak amplitude of peaks $N_1P_2$, and $P_2N_2$ [$F$'s (3, 30) $\geq 4.12$, $p$'s $\leq 0.0391$].
AROCLOR 1254-INDUCED HEARING DEFICITS

When the 1-kHz stimulus was used, only the latency of peak $P_4$ was significantly altered by exposure to A1254 (Fig. 1, Table 1). A significant main effect of treatment was observed [$F(3, 30) = 5.93, p = 0.0027$], and significant treatment-related effects were observed for the 65-dB SPL stimulus [$F(3, 30) = 5.27, p = 0.0048$]. For the 1-kHz, 65-dB SPL stimulus, 8 mg/kg/day A1254 increased peak $P_4$ latency compared with the 0 and 1 mg/kg/day doses. No significant differences from controls (all $p's \geq 0.05$) were observed for the 1-kHz, 80-dB SPL stimulus.

No significant differences from controls (all $p's \geq 0.05$) for any peak latency were observed for the 4-, 16-, or 32-kHz stimuli at either stimulus intensity (Figs. 2–4, Table 1).

The interpeak latency between peaks $P_4$ and $P_{1A}$ was not significantly altered by exposure to A1254 compared with

FIG. 3. Average BAER waveforms ($n = 6–11$ rats/waveform) at 16 kHz (80 and 40 dB SPL) after developmental exposure to 0, 1, 4, or 8 mg/kg/day Aroclor 1254. Exposure to Aroclor 1254 did not alter BAER waveforms at 16 kHz.

FIG. 4. Average BAER waveforms ($n = 6–11$ rats/waveform) at 32 kHz (80 and 40 dB SPL) after developmental exposure to 0, 1, 4, or 8 mg/kg/day Aroclor 1254. Exposure to Aroclor 1254 did not alter BAER waveforms at 32 kHz.
controls (all p's ≥ 0.05) for any of the stimuli (Figs. 1–4, Table 1).

Colonic Temperature

There were no significant differences in colonic temperature between the different A1254 treatment groups (p > 0.05). The mean ± SE colonic temperatures for the 0, 1, 4, and 8 mg/kg/day doses were 38.0 ± 0.2, 38.3 ± 0.1, 37.9 ± 0.2, and 37.8 ± 0.3°C, respectively.

DISCUSSION

Developmental exposure to A1254 decreased BAER peak amplitudes and increased peak P4 latency (1 kHz, 65 dB SPL). The decreases in peak amplitudes were dose related and occurred at the lower frequencies (1 and 4 kHz), but not at the higher frequencies (16 and 32 kHz). The greater severity of auditory dysfunction (elevated reflex modification threshold) at lower frequencies is consistent with a previous report (Goldey et al., 1995b). The present data indicate a LOEL of 1 mg/kg/day for the decrease in peak P1A, amplitude using an 1-kHz tone presented at 80 dB SPL (Figs. 1, 6). It is important to note that the 1 mg/kg/day dose of A1254 did not increase postnatal mortality or alter body weight gain (Goldey et al., 1995b).

The changes in BAERs produced by developmental exposure to A1254 are consistent with peripheral auditory dysfunction. Peak P1 (P1A, and P1B) is believed to be generated in the auditory nerve (Chen and Chen, 1991; Møller et al., 1988; Starr and Zaaroor, 1990). The observed decreases in the amplitudes of peaks P1A, P1A,N1, and P1B,N1 following A1254 exposure suggest a reduced level of activity in the auditory nerve following stimulation with a 1-kHz tone. This reduced activity may result from damage to the auditory nerve itself or more peripheral auditory structures (Achor and Starr, 1980; Chen and Chen, 1991). Decreases in the amplitude of subsequent BAER peaks may result from peripheral dysfunction or damage to the retrocochlear generators themselves (Achor and Starr, 1980; Chen and Chen, 1991; Fullerton and Kiang, 1990; Wada and Starr, 1983). The amplitudes of peaks N1,P3, and P2N2 were decreased by developmental A1254 exposure when tested with the 4-kHz, 60-dB SPL stimulus. The origin of peak P3 is hypothesized to be at the level of the cochlear nucleus (Buchwald, 1983; Chen and Chen, 1991; Møller and Jannetta, 1985, 1986). The decreased amplitudes of these peaks may be related to reduced afferent input via the auditory nerve or to damage to the cochlear nucleus.

Increases in the latency of peak P4 were observed when the 1-kHz (65-dB SPL) stimulus was used. Because peak P4 may be generated at the level of the lateral lemniscus in animals (Buchwald, 1983; Fullerton and Kiang, 1990; Henry, 1979), the increase in peak P4 latency may be related either to a slightly increased latency of the afferent input or to changes in the propagation of the auditory signal at the level of the brainstem. These possibilities remain to be differentiated, as the peripheral peaks (e.g., P1A) were too small to be quantified at 65 dB SPL for the 1-kHz stimulus in our current testing apparatus; however, two lines of evidence suggest that the increase in peak P4 latency is not due to retrocochlear damage. First, at the lowest dosage (1 mg/kg/day) the only changes we observed were the decreased amplitudes of peaks P1A,N1 and P1B,N1, which are generated peripheral to the lateral lemniscus. Second, analysis of the
interpeak latency between peaks $P_4$ and $P_2$ indicated no significant changes due to A1254 exposure compared with controls for any stimulus condition (data not shown).

It is unlikely that the increase in peak $P_4$ latency is related to hypothermia (Chen and Chen, 1992; Janssen et al., 1991; Katbanna et al., 1993), as no significant differences in colonic temperature between the various groups were observed. Overall, these data are consistent with the hypothesis that developmental exposure to A1254 produces damage to the peripheral auditory system, possibly at the level of the cochlea and/or auditory nerve. Damage to auditory structures at the level of the brainstem, however, cannot be dismissed.

Although this study used a subset of the animals previously tested (Goldey et al., 1995b), there were several minor differences in the dose- and frequency-related changes in BAERs and elevations in auditory thresholds. Decreases in peak amplitudes were observed at both 1 and 4 kHz (Figs. 1, 2, 5, 6). In contrast, auditory thresholds were significantly elevated only at 1 kHz when measured using reflex modification audiometry (Goldey et al., 1995b). Additionally, we observed significant changes in peak $P_{1A}N_1$ amplitude at doses as low as 1 mg/kg/day A1254 (Figs. 1, 6), whereas auditory thresholds were elevated only at the 4 and 8 mg/kg/day treatments (Goldey et al., 1995b). These differences may be related to a more direct measure of peripheral auditory function provided by the BAER, different amounts of variability in the data between the two studies, and/or the increased age of the animals at the time of this experiment (e.g., increased treatment effects with aging).

Two stimulus intensities were used to characterize the changes in BAERs produced by A1254 because these animals have shown elevated low-frequency thresholds (Goldey et al., 1995b) which may be potentially detected easier at lower stimulus intensities. The multiple stimulation intensities also served to demonstrate that we could readily quantify changes in BAERs of the magnitude produced by the different stimulus conditions. Decreases in BAER peak amplitudes were observed at the 80-dB SPL intensity ($P_{1A}N_1$, $P_{1B}N_1$, $N_1P_2$, $P_2N_2$; Figs. 1, 6) for the 1-kHz stimulus. The apparent lack of effects at 65 dB SPL (1 kHz), excepting the increase
in peak P4 latency, is likely to be due to the small responses recorded under these conditions which limited the range of effects that could be detected. In contrast, decreases in peak amplitudes were observed at 60 dB SPL (N,P11, and P12; Figs. 2, 5) for the 4-kHz stimulus. The greater incidence of changes at 60 dB SPL suggests that the 80-dB SPL intensity may have overcome the auditory dysfunction produced by A1254, possibly via loudness recruitment (Gulick et al., 1989). No alterations in BAERs were observed at either stimulus intensity for the 16- or 32-kHz stimuli, nor was there any indication of distortion of the BAER waveform, that would suggest treatment-related changes at these frequencies. Additionally, there was no change in auditory threshold at 16 or 32 kHz when these same animals were tested using reflex modification audiometry (Goldey et al., 1995b). Therefore, it is unlikely that treatment-related effects were missed by the 40-dB SPL stimulus at these frequencies.

Hypothyroidism has been previously associated with peripheral auditory dysfunction (Goldey et al., 1995b,c; Hébert et al., 1985; Henley and Rybak, 1995; Uziel, 1986; Uziel et al., 1980, 1985a,b; Van Middlesworth and Norris, 1980), and may be an underlying event in the auditory deficits produced by developmental exposure to A1254. Propylthiouracil-induced hypothyroidism has been shown to decrease the cochlear microphonic and compound action potential recorded from the round window in rats (Uziel et al., 1980), and increase the latency of wave I (P1A and P1B) of the BAER (Hébert et al., 1985). These alterations in peripheral auditory electrophysiology and morphological changes in cochlear structures have been shown to be attenuated by postnatal administration of thyroxine (T4) (Hébert et al., 1985; Uziel et al., 1985a,b). Developmental exposure to A1254 reduced circulating concentrations of thyroxine (free T4) over PNDs 7–40 and of triiodothyronine (total T3) over PNDs 21 and 30 (Goldey et al., 1995b). This A1254-induced perinatal hypothyroidism resulted in a loss of low-frequency behavioral auditory thresholds (Goldey et al., 1995b). Cochlear development proceeds in a basal (high frequencies) to apical (low frequencies) direction, over a period that includes PNDs 1–36 (Millner, 1991; Puel and Uziel, 1987; Roth and Bruns, 1992a,b; Rubel, 1978). Thus, these animals had reduced levels of circulating thyroid hormones (Goldey et al., 1995b) at a time when the apical cochlear function is developing (Müller, 1991), resulting in a low-frequency auditory dysfunction (Goldey et al., 1995b) (and this report).

In summary, this study provides corroborative evidence for a selective low- to mid-frequency auditory dysfunction produced by developmental exposure to A1254. The decreased amplitude of the early BAER peaks indicates that the deficit may exist at the level of the cochlea and/or auditory nerve. The changes in auditory function may be related to neonatal hypothyroidism produced by A1254. Future experiments should investigate histomorphological alterations in the cochlea and auditory nerve produced by A1254.

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* Dose in mg/kg/day.
* Stimulus intensity in dB peak SPL.
* Not quantified due to inadequate SNR.
* Significantly different from 0 and 1 mg/kg/day doses (Tukey-Kramer test).
and implementation of the software and hardware that were used in this experiment. The assistance of Ms. K. Micor in preparing the graphs is greatly appreciated.

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