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

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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).


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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. 11097-69-1; 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 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 (Braintrace Scientific Inc., Brainerid, 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; Physistep Instruments, Inc., Clifton, NJ) was inserted approximately 8 cm rectally and connected using a shielded and grounded wire to a thermometer (Model TH-8, Physistep 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 mm before stimulation.

Auditory stimuli were simulated filtered clicks centered at 1, 4, 16, or 32 kHz. Each intensity was presented at two intensities (peak dB SPL; re: 20 μPa): 65 and 80 for 1 kHz, 60 and 80 for 4 kHz, and 40 and 80 for 16 and 32 kHz. The lower stimulus intensity was chosen such that easily recognizable waveforms (above threshold) would be recorded from control animals (1 and 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 was output 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 EAS10TH400A, 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 across the different dose groups.

The evoked responses were amplified 10,000× and bandpass filtered (0.1–20 kHz; 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 P1 and P2, 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 & Kjaer Measuring Amplifier (Model 2636) with a 0.635-cm microphone (Model 4135, Bruel & Kjaer, 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 two times 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 a between-subject factor, and stimulation frequency and intensity were within-subject factors. The data for each frequency were analyzed separately because the different intensities 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 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).
At 1 kHz, the amplitude of virtually every portion of the BAER was decreased by A1254 exposure (Figs. 1, 6). Significant main effects of treatment, or a treatment × intensity interaction, were observed for the amplitudes of peaks P_{1A}, P_{1A}N_1, P_{1B}N_1, N_1P_2, and P_2N_2 [F's(3, 30) ≥ 5.92, p's ≤ 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 × intensity interaction, were observed for the peak-to-peak amplitude of peaks N_1P_2, and P_2N_2 [F's(3, 30) ≥ 3.16, p's = 0.0391]. No significant differences in peak amplitude were observed for the 1-kHz stimulus at 65 dB SPL; however, at 80 dB SPL (Fig. 6), there was a dose-dependent decrease in the baseline-to-peak amplitude of peak P_{1A} [F(3, 30) = 3.52, p = 0.0268]. No significant mean differences were indicated by the Tukey–Kramer test. Also at 80 dB SPL (Fig. 6), there were dose-dependent decreases in the peak-to-peak amplitudes of peaks P_{1A}N_1, P_{1B}N_1, N_1P_2, and P_2N_2 [F's(3, 30) ≥ 3.16, p's = 0.0391].

**FIG. 1.** Average BAER waveforms (n = 6–11 rats/waveform) at 1 kHz (80 and 65 dB SPL) after developmental exposure to 0, 1, 4, or 8 mg/kg/day Aroclor 1254. Exposure to Aroclor 1254 decreased peak amplitudes at 1 kHz. In all figures, the waveforms are plotted with positivity upward. Waveforms are displaced vertically for visual clarity rather than representing different DC response levels between conditions. Only peaks that had a SNR ≥ 2 were quantified, and in all figures these peaks are labeled on the appropriate control waveforms. See Results and other figures for details of Aroclor 1254-related effects on specific portions of BAERs.

**FIG. 2.** Average BAER waveforms (n = 6–11 rats/waveform) at 4 kHz (80 and 60 dB SPL) after developmental exposure to 0, 1, 4, or 8 mg/kg/day Aroclor 1254. Exposure to Aroclor 1254 decreased peak amplitudes at 1 kHz. In all figures, the waveforms are plotted with positivity upward. Waveforms are displaced vertically for visual clarity rather than representing different DC response levels between conditions. Only peaks that had a SNR ≥ 2 were quantified, and in all figures these peaks are labeled on the appropriate control waveforms. See Results and other figures for details of Aroclor 1254-related effects on specific portions of BAERs.
AROCLO1 1254-INDUCED HEARING DEFICITS

123

When the 1-kHz stimulus was used, only the latency of peak P, 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, 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, and P, was not significantly altered by exposure to A1254 compared with

\[ F(3, 30) = 5.93, p = 0.0027 \]. At 60 dB SPL (Fig. 5), the amplitudes of these BAER peaks were decreased by A1254 exposure \[ F'(3, 30) = 5.05, p' = 0.0060 \]. The amplitude of peak N,P was decreased by 4 and 8 mg/kg/day A1254 compared with the 0 and 1 mg/kg/day doses. Peak P, amplitude was decreased by 4 and 8 mg/kg/day A1254 compared with controls. There were no significant differences from controls produced by treatment with A1254 for the peak-to-peak amplitude of peak P, or P, or the baseline-to-peak amplitude of peak P,A or P, at 60 dB SPL. Additionally, treatment with A1254 did not produce significant differences from controls for any peak amplitude when the animals were tested with the 4-kHz, 80-dB SPL stimulus.

There were no significant (all \( p \)'s \( \geq 0.05 \)) treatment-related changes in any portion of the BAER waveform at 16 or 32 kHz at either the 40- or 80-dB SPL stimulus levels (Figs. 3–6).

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 of 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 P4 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 P4 (P1A and P1B) is believed to be generated in the auditory nerve (Chen and Chen, 1991; Möller 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 N1P3 and P3N2 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; Möller 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 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

FIG. 5. Group means (n = 6-11 rats/group) of the peak-to-peak amplitude of peaks N1P2 and P2N2 for the low-intensity stimuli at 1 (65 dB SPL), 4 (60 dB SPL), 16 (40 dB SPL), and 32 (40 dB SPL) kHz after perinatal exposure to 0, 1, 4, or 8 mg/kg/day Aroclor 1254 (*different from 0 mg/kg/day; + different from 1 mg/kg/day). Peak P2N2 was not quantified at 1 kHz due to an inadequate SNR. See Results for details.
interpeak latency between peaks P4 and P2 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 P4 latency is related to hypothermia (Chen and Chen, 1992; Janssen et al., 1991; Katbamna 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 P1A,N1 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 (P1A,N1, P1B,N1, N1,P2, P2,N2; Figs. 1, 6) for the 1-kHz stimulus. The apparent lack of effects at 65 dB SPL (1 kHz), excepting the increase...
HERR, GOLDEY, AND CROFTON

TABLE 1
Peak Latencies of BAER Peaks P_{1A} and P_{4} and the Peak P_{1A}P_{4} Interpeak Latency (msec + SE)

<table>
<thead>
<tr>
<th>Peak</th>
<th>Dose^a</th>
<th>1 kHz</th>
<th>4 kHz</th>
<th>16 kHz</th>
<th>32 kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65 dB</td>
<td>80 dB</td>
<td>60 dB</td>
<td>80 dB</td>
<td>40 dB</td>
</tr>
<tr>
<td>P_{1A}</td>
<td>NA^c</td>
<td>3.17 ± 0.11</td>
<td>2.48 ± 0.02</td>
<td>2.19 ± 0.01</td>
<td>1.93 ± 0.01</td>
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<tr>
<td></td>
<td>NA^c</td>
<td>3.28 ± 0.04</td>
<td>2.60 ± 0.10</td>
<td>2.20 ± 0.02</td>
<td>1.91 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>NA^c</td>
<td>3.42 ± 0.13</td>
<td>2.71 ± 0.17</td>
<td>2.22 ± 0.03</td>
<td>1.91 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>NA^c</td>
<td>3.23 ± 0.03</td>
<td>2.22 ± 0.03</td>
<td>2.28 ± 0.06</td>
<td>1.92 ± 0.02</td>
</tr>
<tr>
<td>P_{4}</td>
<td>NA^c</td>
<td>2.56 ± 0.13</td>
<td>2.19 ± 0.05</td>
<td>2.22 ± 0.04</td>
<td>2.24 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>NA^c</td>
<td>2.43 ± 0.08</td>
<td>2.12 ± 0.03</td>
<td>2.14 ± 0.02</td>
<td>2.25 ± 0.03</td>
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<tr>
<td></td>
<td>NA^c</td>
<td>2.78 ± 0.15</td>
<td>2.47 ± 0.12</td>
<td>2.28 ± 0.05</td>
<td>2.30 ± 0.04</td>
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<tr>
<td></td>
<td>NA^c</td>
<td>2.86 ± 0.13</td>
<td>2.58 ± 0.20</td>
<td>2.19 ± 0.04</td>
<td>2.28 ± 0.06</td>
</tr>
<tr>
<td>P_{1A}P_{4}</td>
<td>6.59 ± 0.21</td>
<td>5.72 ± 0.10</td>
<td>4.67 ± 0.06</td>
<td>4.40 ± 0.05</td>
<td>4.18 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>6.52 ± 0.10</td>
<td>5.71 ± 0.13</td>
<td>4.71 ± 0.13</td>
<td>4.34 ± 0.02</td>
<td>4.16 ± 0.03</td>
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<tr>
<td></td>
<td>7.06 ± 0.19</td>
<td>6.19 ± 0.18</td>
<td>5.18 ± 0.20</td>
<td>4.50 ± 0.06</td>
<td>4.22 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>7.32 ± 0.06^d</td>
<td>6.09 ± 0.13</td>
<td>4.86 ± 0.06</td>
<td>4.47 ± 0.09</td>
<td>4.21 ± 0.07</td>
</tr>
</tbody>
</table>

^a Dose in mg/kg/day.
^b Stimulus intensity in dB peak SPL.
^c Not quantified due to inadequate SNR.
^d Significantly different from 0 and 1 mg/kg/day doses (Tukey–Kramer test).

in peak P_{4} 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_{P_2} and P_{2N_2}; 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. Propylthio-uracil-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 (P_{1A} and P_{1B}) 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 (T_{4}) (Hébert et al., 1985; Uziel et al., 1985a,b). Developmental exposure to A1254 reduced circulating concentrations of thyroxine (free T_{4}) over PNDs 7–40 and of triiodothyronine (total T_{3}) 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 (Müller, 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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