Changes in auditory brainstem response in rats chronically exposed to carbon disulfide

Mamoru Hirata1, Yasutaka Ogawa2, Akira Okayama3 and Shigeru Goto4

1 Department of Occupational Health, Osaka Prefectural Institute of Public Health
2 Department of Hygiene, Jikei University School of Medicine
3 Department of Health Care and Administration, Shiga University of Medical Science
4 Nara University of Education

Abstract. The chronic effect of carbon disulfide (CS2) on the central nervous system (CNS) was studied by examining auditory brainstem responses (ABRs) in female rats (Jcl Wistar) exposed to 200 ppm or 800 ppm CS2 by inhalation, 6 h a day, 5 days a week, for 15 weeks. Two modes of ABRs evoked by clicks at 61 and 96 dB sound pressure levels (61 dB-ABR and 96 dB-ABR) were recorded during the exposure and for 6 weeks afterwards. Three main components (I, III and V) of ABRs were analyzed from the latencies and differences between latencies of them (interpeak latencies, IPL I–III, IPL III–V and IPL I–V). The latencies of the three components and IPLs of 96 dB-ABR in rats group exposed to 800 ppm of CS2 were significantly delayed during the exposure period. The delay of latency of component V and IPL III–V and I–V tended to increase with exposure time. At 61 dB-ABR, the changes in the latency of component V, IPL III–V and I–V resembled those at 96 dB-ABR. For the rats group exposed to 200 ppm CS2, the latency of component I, IPL III–V and I–V at 96 dB-ABR were delayed significantly but transiently during the exposure period. For both groups, recovery from the latencies of the three components and IPLs of ABR was observed by the end of the recovery period. The delayed latencies of ABR observed in rats exposed to 800 ppm CS2 suggested a conduction dysfunction in the brainstem due to CS2 exposure. The transient delay of the parameters in the rats group exposed to 200 ppm CS2 was considered to represent a slight conduction dysfunction.

Key words: Carbon disulfide – Rats – Auditory brainstem response – Conduction function – Central nervous system

Introduction

Exposure to carbon disulfide (CS2) often causes acute and chronic disorders of the central nervous system (CNS), which are referred to as “CS2 encephalopathy” (Seppäläinen and Haltia 1980). At the average workplace exposure level of less than 10 ppm in Japan, only mild symptoms and signs of encephalopathy are observed. However, unspecific symptoms like general fatigue, headache and sleep disturbances frequently occur. Electroencephalographical changes among workers chronically exposed to CS2 have been reported by Seppäläinen and Tolonen (1974), Roukova (1975), Ginsburg and Pershai (1976), and Stybrova (1977).

Recently, sensory evoked potential techniques, which are sensitive enough to detect CNS functions, have been developed. The auditory brainstem response (ABR), a type of sensory evoked potential, essentially reflects CNS function. Rebert et al. (1982) applied the ABR technique to a neurotoxicological study of organic solvents in animal experiments. In 1987, Hirata, one of the present authors, observed significant changes in ABR among rats administered 2,5-hexanedione (a major metabolite of n-hexane). However, only a few studies on “CS2 encephalopathy” have been conducted using the ABR technique.

In the present study, in order to detect the effect of chronic CS2 exposure on CNS, we used ABRs as indicators of the conduction function in CNS using subcutaneous needle electrodes which enabled repeated measurements of ABR in rats. The effects of the exposure levels on CNS function represented by the conduction function and the time course of changes in ABR during the exposure and recovery after the end of exposure are discussed. Since this study was designed for a long time schedule including the exposure period and the recovery period, we employed rats because they are easy to handle and have small body weight gain. We selected two exposure levels of CS2, that is, 200 ppm as the threshold level for effects on the nervous system and 800 ppm as the level on which the effects are clearly evident.
Materials and methods

Experimental animals. Female Wistar rats, 11 weeks old and weighing 160–180 g, were purchased from Clea Japan (Tokyo, Japan) and kept at constant temperature (22–23 °C) with 12 h illumination per day. They were permitted free access to food (standard laboratory chow) and tap water. The rats were divided into three groups of 12 and exposed to 800 ppm, 200 ppm CS₂ and fresh air, respectively (800 ppm group, 200 ppm group and control group, respectively), in constant flow chambers (Okayama et al. 1987), 6 h a day, 5 days a week for 15 weeks. The exposure was stopped before the measurement of ABRs at week 15 of the exposure period, and then the rats were allowed a 6-week recovery period. The concentrations of CS₂ in the exposure chambers were monitored every morning and afternoon using a colorimetric method (McKee 1941) with a slight modification. The variation in the CS₂ concentration in the exposure chambers was within 5% of the fixed concentration. The body weights of the rats were measured on day 6 of each week.

ABR measurement procedure. ABR was measured before the exposure, every 3 weeks during the exposure period, and in weeks 2 and 6 after the end of exposure. All measurements were performed on days 6 and 7 of each week. Rats were anesthetized with pentobarbital (20–25 mg/kg) intraperitoneally and kept warm with a disposable body warmer during measurement. The scalp was punctured subcutaneously at the midpoints of the ears and eyes with stainless steel needles (0.34 mm in diameter) that served as active and reference electrodes, respectively. A clip electrode at the right ear served as the ground. Alternative clicks of 0.1 ms duration with 61 and 95 dB sound pressure level (61 dBSPL and 96 dBSPL) were given monaurally to the left ear through an electrodynamic type earphone using a sound stimulator (Model ST-5, Medelec Co., UK). ABRs evoked by 61 dBSPL and 96 dBSPL intensity clicks were called 61 dB-ABR and 96 dB-ABR, respectively. Responses were amplified by an amplifier (Model AVB-9, Nihon Kohden Co., Japan) with bandpass of 50 Hz to 10 kHz. The 1024 or 2048 responses were averaged by two channel averagers (Model 7SO7, Nippon Denki San-ei Co., Japan) with the measurements being repeated at least twice to confirm the stability of the ABR components. The averaged ABR was drawn on paper using an X–Y recorder (Model 3036, Yokogawa Electric Co., Japan).

Statistical analysis. Analysis of variance (ANOVA) with multiple comparison by Scheffe’s method was used to compare the data from each measurement of the exposed and control groups.

Results

Changes in the mean body weight of the three groups are shown in Fig. 2. Significant changes in body weight gain were observed from week 3 of the exposure period [F (2,33) = 6.29, p = 0.005] to the end of the experiment [F (2,17) = 9.41, p = 0.002]. Significant changes in body weight gain in the 800 ppm group compared with the control group were observed from week 3 of the exposure period (p <0.01) to the end of the experiment (p <0.01), while no significant changes were observed in the 200 ppm group.

In the exposed rats, no apparent symptoms of CS₂ intoxication (paralysis of hindpaw, changes in activity, etc.) were observed throughout the experiment.

Adequate numbers of rats were available throughout the experiment, but six rats did not recover from anesthesia (one rat from the 200 ppm in week 3, one rat from the 200 ppm group in week 6, two rats from the 200 ppm group in week 12 and two rats from the 800 ppm group in week 15, respectively). In week 2 in the recovery period, five rats from the 800 ppm group and five rats from the control group were sacrificed for pathological examination.