Trimethyltin-induced loss of NMDA and kainate receptors in the rat brain

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Summary. Adult rats exposed acutely to trimethyltin (TMT) manifest a number of behavioral alterations, in conjunction with neuronal degeneration in the limbic system. In the present study, changes in ³H-TCP binding to N-methyl-D-aspartate (NMDA) receptors and ³H-kainic acid (KA) binding to kainate receptors were studied by autoradiographic methods following TMT exposure (8 mg/kg, i.p.) in adult Sprague Dawley rats. No significant alterations were found at 4 hours after exposure. An extensive loss of ³H-TCP and ³H-KA binding was seen in the hilar region of the CA3 field at 2 and 12 weeks after TMT exposure. Also, the ³H-TCP binding was decreased in piriform cortex and in striatum. Thus, TMT exposure leads to a major and regional selective loss of NMDA and kainate receptors in the limbic system, alterations that may be involved in the neuropathology and behavioral sequelae of TMT toxicity.

Keywords: Amino acids – Trimethyltin – Glutamate system – NMDA receptors – Kainate receptors – Hippocampus

Abbreviations: TMT: trimethyltin; NMDA: N-methyl-D-aspartate; KA: Kainic acid; TCP: N-(1-2-thienylcyclohexyl)-3,4-piperidine

Introduction

Organotin compounds have widespread industrial and agricultural applications; for example, lower molecular weight trialkyltins are used as stabilizers of plastics, as chemosterilants and as biocides (Piver, 1973). Accidental exposure to the alkyltin trimethyltin (TMT) in man has been reported to cause anorexia, mental confusion, rage reactions, epileptic seizures and depression as well as memory loss (Fortemps et al., 1978; Ross et al., 1981). In adult rats, acute TMT intoxication produces a behavioral syndrome, which in the early period after
exposure includes aggression (Brown et al., 1979), hyperirritability, tremor and convulsive episodes (Dyer et al., 1982). Locomotor hyperactivity has been shown to develop several days after TMT exposure. In addition, a number of deficits in cognitive tasks have been found to occur following TMT exposure. Thus, an impairment in learning and memory has been demonstrated in the radial maze (Walsh et al., 1982), in the Hebb-Williams maze (Swartzwelder et al., 1982), in the Morris maze and in passive avoidance behavior (Earley et al., 1990; Hagan et al., 1988). A hallmark of TMT neurotoxicity in rats is a loss of neurons in the hippocampal CA3 field of Ammon's horn, although other limbic regions may be affected as well, e.g. the amygdaloid nuclei, the piriform/entorhinal cortex as well as pyramidal cells in the neocortex (Bouldin et al., 1981; Brock and O'Callaghan, 1986; Brown et al., 1979; Chang and Dyer, 1983; Woodruff and Baisden, 1990). The neurotoxic mechanisms of TMT are not well understood, although it has been suggested that damage of neurons occur due to hyper-stimulation, possibly through dysfunction in the regulation of excitatory amino acid neurotransmission in the brain (Chang, 1986).

In the present study we have investigated the effects of a single dose of TMT on NMDA and kainate receptors over time in adult rats. The receptor binding was determined in brain sections by in vitro autoradiographic techniques using $^3$H-TCP and $^3$H-KA as ligands.

Materials and methods

Animals and treatment

Male adult Sprague-Dawley rats (approx. 200 g; B&K Universal AB, Sollentuna, Sweden) were housed in air-conditioned rooms with constant temperature and a standardized light/dark schedule (12/12 h; light on at 06.00 h and off at 18.00 h). Food and water were supplied ad libitum. Trimethyltin chloride (TMT, Heraeus) was injected intraperitoneally (i.p.) in a dose of 8 mg/kg body weight. The animals were sacrificed at 4h, 2 and 12 weeks after TMT administration by decapitation. The brains were rapidly removed and frozen on dry ice and stored at $-70 \degree C$ until sectioning. The brains were cut in a cryostat (Leitz, Germany) at $-15 \degree C$ in 14 µm frontal sections from the dorsal hippocampus and hippocampus/mesencephalon, levels 30 and 37 according to the rat brain atlas of Swanson (Swanson, 1992). The sections were collected on gelatin coated glass slides and stored at $-70 \degree C$, until the autoradiographic experiments were performed. Sections from paraformaldehyde-perfused animals were also obtained and stained with cresyl violet.

$^3$H-TCP and $^3$H-KA in vitro autoradiography

The sections were dried in room temperature for at least 120 min before preincubation. TCP autoradiography was performed according to Maragos et al. (1986). The sections were preincubated for 30 min at $4 \degree C$ in Tris-acetate buffer (50 mM, pH = 7.4) including 4 mM CaCl$_2$. The sections were dried again followed by an incubation at room temperature for 45 min in the Tris-acetate buffer containing 1 mM magnesium acetate and 20 nM $^3$H-TCP in the presence or absence of 20 µM phenycyclidine (PCP) to define non-specific binding. The incubation was terminated by washing the sections in ice-cold Tris-acetate buffer for $3 \times 1$ min. The sections were then dried under a stream of cool air and subsequently apposed to $^3$H-sensitive film (Amersham, England) and stored at $-20 \degree C$ in X ray cassettes for 8 weeks.

KA autoradiography was performed according to Patel et al. (1986). The sections were preincubated for 15 min at 30°C in Tris-acetate buffer (50 mM, pH = 7.2). The sections were