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Reversal of lindane-induced impairment of step-down passive avoidance and oxidative stress by neurosteroids in rats

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Abstract

Neurosteroids (NS) are recognized as important modulators of functioning of the nervous system. Lindane, an organochlorine pesticide has been shown to adversely affect memory and induce oxidative stress on both acute and chronic exposure. The present study was designed to explore the modulation of effects of lindane over cognitive function by progesterone (PROG), pregnenolone sulfate (PREG-S) and 4′-chlorodiazepam (4CD). Cognitive function was assessed using step-down latency (SDL) on a passive avoidance apparatus and transfer latency (TL) on a plus maze. Oxidative stress was assessed by examining brain malondialdehyde (MDA) and non-protein thiol (NP-SH) levels. A significant reduction in SDL was found for the lindane treated group at weeks 6 and 7 as compared to control ($p < 0.001$). One-week treatment by PREG-S or 4CD antagonized the effect of lindane on SDL. PROG failed to modulate the effect of lindane on SDL. Lindane caused a significant prolongation of TL as compared to control ($p < 0.001$) from second week onwards. One-week administration of PROG, PREG-S or 4CD was unable to reverse this prolongation of TL. Lindane produced a statistically significant increase in the brain MDA levels ($p < 0.001$) and significant decrease in the brain NP-SH levels ($p < 0.001$). Treatment with PREG-S and 4CD attenuated the effect of lindane on MDA ($p < 0.001$) and NP-SH levels. PROG failed to influence oxidative stress induced by lindane. Results of the present study thus show that some NS have potential in reversing cognitive dysfunction and oxidative stress induced by toxicants like lindane in the brain.

Keywords: Lindane; Neurosteroids; Pregnenolone sulfate; Progesterone; 4′-Chlorodiazepam

1. Introduction

Neurosteroids (NS) are steroids that are newly synthesized from cholesterol and are present in the nervous system even after removal of peripheral steroidogenic gland (Baulieu and Robel, 1990; Robel and Baulieu, 1994; Stoffel-Wagner, 2001). Occurring as unconjugated steroids, sulfated esters and fatty acid esters of steroids, they are involved in the control of metabolic, behavioral and psychical processes including cognition, stress, anxiety, sleep, etc. Examples of known NS include progesterone (PROG), pregnenolone (PREG), pregnenolone sulfate (PREG-S), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), allopregnanolone (AP), tetrahydrodeoxycorticosterone (THDOC), etc. (Jo et al., 1989; Baulieu and Robel, 1990; Majewska, 1992; Kulkarni and Reddy, 1995; Djebaili et al., 2004). 4′-Chlorodiazepam (4CD) not a NS in its own

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right, increases the production of NS by acting through mitochondrial diazepam binding inhibitor receptor complex (MDRC) (Korneyev et al., 1993).

In the last few decades a lot of emphasis has been laid on the role of NS in the functioning of the nervous system. They have been implicated in various functions including cognition. Notable among cognition are their role in neuroprotection and reinforcement of long-term memory, active avoidance behavior and learning. Previous workers have noticed a general trend toward decreased levels of PREG-S, DHEA-S, PROG and AP in Alzheimer's disease patients' brain (Stoffel-Wagner, 2001; Weill-Engerer et al., 2002). NS have been correlated with improvement of memory retention in foot-shock active avoidance training. PREG-S was reported to facilitate memory and AP was shown to possess rewarding properties. As little as 150 molecules of PREG-S significantly enhance post-training memory processes when injected into the amygdale of rats (Baulieu and Robel, 1990; Flood et al., 1992; Finn et al., 1997; Reddy and Kulkarni, 1998; Vongher and Frye, 1999; Johansson et al., 2002). PROG has been shown to have a neuroprotective role on learning and memory impairment and hippocampus damage in rats (Vongher and Frye, 1999). AP, PREG-S, PROG and MDR ligand 4CD possess neuroprotective activity in hypoxic stress models in rats. Further, neuroprotection after injury has been demonstrated in contusion of prefrontal cortex and spinal trauma with PROG, AP and PROG, respectively (Reddy and Kulkarni, 1997; Thomas et al., 1999; Djebaili et al., 2004).

It is now well recognized that NS can mediate their effects through genomic mechanisms, through steroid receptors and through nongenomic mechanisms via γ-amino butyric acid-A (GABA-A), nicotinic and muscarinic acetylcholine, sigma (σ), *N*-methyl-D-aspartate (NMDA), serotonergic, kainate, glycine, neuropeptide receptors, voltage gated Ca2+ channel, microtubule-associated protein 2 (Kulkarni and Reddy, 1995; Harrison and Simmonds, 1984; Wu et al., 1990; Paul and Purdy, 1992; Valera et al., 1992; Fahey et al., 1995; Monnet et al., 1995; Czlonkowska et al., 1999, 2001, 2003; Birzniece, 2004; Fontaine-Lenoir et al., 2006).

4CD increases the synthesis of NS by increasing the delivery of cholesterol to the biosynthetic pathway. Post-training intra-amygdala administration of picrotoxin or 4CD enhances retention (Izquierdo et al., 1991). Post-training i.p. (2.0 or 5.0 mg/kg), i.c.v. (2.5 μg/rat), or intra-amygdala (1.6–40 ng/amygdala) administration of 4CD causes memory facilitation of step-down inhibitory avoidance in rats (Da Cunha et al., 1991).

NS are now being proven to have a definite role in the antioxidant defenses of central nervous system (Bucolo et al., 2005; Tunez et al., 2005). PROG has been indicated to have an inhibitory action on lipid peroxidation. In cerebral contusion model in rats, brains of progesterone treated rats contained approximately one-third of the 8-isoprostaglandin F2 alpha, a marker of lipid peroxidation than in control (Roof et al., 1997).

Lindane, an organochlorine pesticide has been shown to produce a number of adverse effects including neurotoxicity. It also reportedly influences the metabolism of pregnenolone (PREG) and progesterone (PROG) in mice ovaries. Lindane inhibits the conversion of cholesterol to PREG by inhibiting the enzyme cytochrome P450 side chain cleavage (P450scc), the rate limiting step in NS biosynthesis (Sircar and Lahiri, 1990). It has further been claimed that lindane inhibits the activity of steroidogenic acute regulatory (StAR) protein, which mediates an important step in hormone-regulated steroidogenesis, the intramitochondrial transfer of cholesterol to the P450scc enzyme (Walsh and Stocco, 2000; Sujatha et al., 2001). Tilson et al. (1987) demonstrated interference with the ability of avoidance response with a single dose of lindane in rats. Desi (1974) found that repeated exposure to lindane increased the number of errors made in a food-reinforced maze. In a study by Griffith and Woolley (1989), the authors when using hypothermia and anorexia as indices of lindane toxicity, demonstrated that toxicity of lindane was ameliorated by diazepam, phenytoin and exacerbated by 4CD. Lindane has also been shown to be a strong oxidant causing free radical generation in tissues including brain through lipid peroxidation (Koner et al., 1998; Sahoo et al., 2000).

Thus, the anti-amnestic, neuroprotective and antioxidant properties of several NS make them a strong contender to reverse the neurobehavioural effects of lindane. Hence, the present study was designed to investigate the effects of PROG, PREG-S and 4CD on lindane-induced modulation of cognitive functions and oxidative stress in rats.

2. Materials and methods

Lindane, PROG and PREG-S were obtained from Sigma chemicals (USA). 4CD was procured from Fluka (USA). All other chemical used were of laboratory grade. Lindane was given per orally (p.o.) with groundnut oil as a vehicle. To limit the weight gain of animals due to oil consumption, the concentration of lindane was maintained so that no animal received more than 0.5 ml of oil per day, anytime during the study period. PROG, PREG-S and 4CD were administered intra peritoneally (i.p.). The vehicle for them was distilled water with two drops of Tween-80 added per 10 ml of suspension. Concentration