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The effect of the steroid sulfatase inhibitor (*p*-O-sulfamoyl)-tetradecanoyl tyramine (DU-14) on learning and memory in rats with selective lesion of septal–hippocampal cholinergic tract

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ABSTRACT

Dehydroepiandrosterone sulfate (DHEAS), is an excitatory neurosteroid synthesized within the CNS that modulates brain function. Effects associated with augmented DHEAS include learning and memory enhancement. Inhibitors of the steroid sulfatase enzyme increase brain DHEAS levels and can also facilitate learning and memory. This study investigated the effect of steroid sulfatase inhibition on learning and memory in rats with selective cholinergic lesion of the septo-hippocampal tract using passive avoidance and delayed matching to position T-maze (DMP) paradigms. The selective cholinergic immunotoxin 192 IgG-saporin (SAP) was infused into the medial septum of animals and then tested using a step-through passive avoidance paradigm or DMP paradigm. Peripheral administration of the steroid sulfatase inhibitor, DU-14, increased step-through latency following footshock in rats with SAP lesion compared to both vehicle treated control and lesioned animals ($p < 0.05$). However, in the DMP task, steroid sulfatase inhibition impaired acquisition in lesioned rats while having no effect on intact animals. These results suggest that steroid sulfatase inhibition facilitates memory associated with contextual fear, but impairs acquisition of spatial memory tasks in rats with selective lesion of the septo-hippocampal tract.

1. Introduction

Neuroactive steroids and neurosteroids play an important role in normal physiology and in the pathogenesis of brain diseases (Kriz, Bicikova, Hill, & Hampl, 2005; Morrow, 2007; Vallee, Mayo, & Le Moal, 2001). Neurosteroids possess both rapid non-genomic as well as slow genomic effects (Akk et al., 2007; Hosie, Wilkins, & Smart, 2007; Kriz et al., 2005; Melcangi, Garcia-Segura, & Mensah-Nyagan, 2008). In contrast to steroids synthesized in classic steroidogenic tissues that activate cytoplasmic receptors, neurosteroids predominantly use signaling pathways common for neuromodulators. It is well known that unconjugated forms of neurosteroids, such as saturated progesterone metabolites 5α-pregnan-3α-ol-20-one (allopregnanolone), its 21-hydroxylated metabolite, androsterone and dehydroepiandrosterone (DHEA) act on $GABA_A$ receptors as positive modulators that along with other events, increase the permeability of chloride ion channels (Hosie, Wilkins, da Silva, Helena, & Smart, 2006; Kriz et al., 2005). Particular sulfated forms of neurosteroids act in the opposite way, as negative $GABA_A$ receptor modulators (Kriz et al., 2005; Twede, Tartaglia, Covey, & Bamber, 2007; Vallee et al., 2001). In addition to modulating $GABA_A$ receptors, several studies have shown that neurosteroids can also interact with the sigma-1 receptor, which is believed to modulate intracellular calcium mobilization and extracellular calcium influx, NMDA-mediated responses, acetylcholine release and alter monoaminergic function (Debonnel & de Montigny, 1996; Maurice, Roman, & Privat, 1996; Monnet, Mahé, Robel, & Baulieu, 1995). Pregnenolone, DHEA and their sulfate esters behave as sigma-1 agonists, while progesterone is a potent sigma-1 antagonist.

Physiological concentrations of DHEA and dehydroepiandrosterone sulfate (DHEAS) are maintained by steroid sulfatase and neurosteroid sulfuryl transferase which are present in the blood and other peripheral tissues, as well as in the brain, (Baulieu, 1997; Kriz et al., 2005; Wolf & Kirschbaum, 1999). Steroid sulfatase (estrone sulfatase, arylsulfatase C; E.C. 3.1.6.2) is a ubiquitous membrane-bound, microsomal enzyme localized mainly in the endoplasmic reticulum and the nuclear envelope of cells. In the brain, DHEAS is cleaved to DHEA via steroid sulfatase, and

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inhibition of the sulfatase has been demonstrated to enhance learning and spatial memory in rats (Li et al., 1993).

Analogues of (*p*-O-sulfamoyl)-N-alkanoyl tyramine such as (*p*-O-sulfamoyl)-tetradecanoyl tyramine, (DU-14), are effective inhibitors of estrone sulfatase activity (Selcer, DiFrancesca, Chandra, & Li, 2007). A single dose of (30 mg/kg) of DU-14 was able to significantly inhibit steroid sulfatase activity within the liver (Baulieu, 1997) and brain (Li, Rhodes, Burke, & Johnson, 1997). In rodents, chronic administration of DU-14 (30 mg/kg for 15 days i.p.) increased plasma and brain concentrations of DHEAS, while decreasing plasma DHEA (Rhodes, Li, Burke, & Johnson, 1997). DU-14 has also been shown, following chronic administration, to reverse amnesia induced by scopolamine (a muscarinic antagonist), and potentiate the reversal of scopolamine-induced amnesia by DHEAS in passive avoidance and Morris water maze memory tests (Howarth, Purohit, Reed, & Potter, 1994; Johnson, Rhodes, Boni, & Li, 1997; Johnson, Wub, Li, & Maher, 2000; Li et al., 1997). Moreover, peripheral administration of DU-14 (30 mg/kg, i.p.) has been shown to increase acetylcholine release in the hippocampus, which is consistent with other studies that found that increased DHEAS levels in the brain augment the levels of ACh within the hippocampus (Rhodes et al., 1997). In the water maze not only did DU-14 reverse the scopolamine induced amnesia, but also enhanced the performance of unimpaired control animals (Johnson et al., 2000).

It has been hypothesized that the memory enhancing effect of steroid sulfatase inhibitors are the result of enhanced cholinergic neurotransmission in brain structures involved in memory such as the hippocampus. If this were the case, one might predict that for animals in which there was loss of cholinergic neurons projecting to the hippocampus, the administration of DU-14 would be relatively ineffective since there would a diminished capacity for neuromodulators such as DHEAS to enhance cholinergic neurotransmission. However, it is possible steroid sulfatase inhibitors may enhance memory utilizing mechanisms that are independent of cholinergic neurotransmission. One possibility is that DU-14 may affect non-cholinergic systems in ways that could compensate for the loss of muscarinic cholinergic activity. If so, then steroid sulfatase inhibitors could potentially be effective for the treatment of cognitive dysfunctions associated with a loss of basal forebrain cholinergic neurons such as in Alzheimer's disease. Whether or not the cognitive enhancing effects associated with DU-14 are due specifically to changes in ACh release in the hippocampus and cortex is currently unknown.

Previous studies have demonstrated that the administration of the cholinergic neurotoxin 192 IgG-saporin (SAP) into the medial septum (MS), produced selective lesion of cholinergic neurons that project from the MS to the hippocampus. Lesion of this tract resulted in a significant reduction in hippocampal ACh and impaired cognitive function as measured by significant delays in acquisition of a DMP T-maze task (Fitz, Gibbs, & Johnson, 2006, 2008; Gibbs & Johnson, 2007; Johnson, Zambon, & Gibbs, 2002).

The intent of the current investigation is to determine the effect of the steroid sulfatase inhibition on learning and memory in rats with a selective cholinergic lesion of the septo-hippocampal tract.

2. Methodology and procedures

2.1. Animal condition

All experiments followed NIH guidelines for the care and use of laboratory animals and were approved by the Duquesne University Institutional Animal Care and Use Committee. All chemicals were purchased through Sigma Inc. (St. Louis, MO) unless stated otherwise. Male Sprague–Dawley rats weighing (275–300 g) were purchased from Hilltop Lab Animal Inc. (Scottdale, PA) and individually housed in a well ventilated, temperature and humidity controlled facility (22–25 °C, 50–75% humidity). A standard 12:12 h light:dark cycle was maintained and rodents had access to standard laboratory rat chow and water *ad libitum*. Animals were allowed a minimum of 5 days to acclimate to the housing conditions, before any experiments were performed. DU-14 was prepared as previously described (Li et al., 1993).

2.2. Animal surgery

Rats weighing approximately 300 g were anesthetized with pentobarbital (50 mg/kg: IP of a 50 mg/ml stock solution, Ovation Pharmaceuticals, Deerfield, IL), shaved and then placed into a stereotaxic frame (Stoelting, Wood Dale, IL). An incision was made exposing the dorsal aspect of the skull and a small hole (2 mm in diameter) was drilled, through which a stainless steel cannula (28 gauge, Plastics One Inc., Roanoke, VA) was lowered into the medial septum (+0.2 mm bregma 0.0 mm lateral, −5.4 mm dorsal ventral). Animals were infused with either 1 μl of vehicle (artificial cerebrospinal fluid (CSF); CMA Inc., North Chelmsford, MA) or SAP (0.20 μg in 1 μl of artificial cerebrospinal fluid; Advanced Targeting Systems, San Diego, CA, Lot #24–87) over 5 min at 0.2 μl/min using a syringe pump (Harvard Apparatus, Holliston, MA). The dose of SAP was selected based on previous studies that demonstrated a substantial loss of cholinergic neurons in the MS with little non-selective damage to GABAergic neurons (Johnson et al., 2002). Following infusion, the cannula was left in place for 5 min to allow for diffusion of the solution into the tissue. The incision was closed, rats were administered Ibuprofen (1 ml/kg IP, of a 10 mg/ml stock solution) and allowed to recover for 14 days prior to behavioral testing.

2.3. Behavioral testing

2.3.1. Passive avoidance testing

To assess passive avoidance memory retention, 14 days following infusion of SAP or artificial CSF into the MS, a Gemini Avoidance System (San Diego Instruments, San Diego, CA) was used in a modified passive avoidance paradigm. The avoidance apparatus consisted of a box (53 × 53 × 32 cm) with 2 compartments connected by an opening with a computer controlled sliding door. The compartment in which the rats were placed was illuminated, while the other compartment remained dark throughout the experiment.

There were three stages of the behavioral testing: acclimation, acquisition, and retention. During acclimation trials rats were allowed to explore the apparatus and the latency period, the time it took for the rat to cross into the dark compartment, was recorded. During the acquisition trials, rats were placed in the lighted compartment. After 5 s of adaptation, the rats were given a maximum trial duration of 5 min to cross to the darkened chamber. If the animal entered the dark compartment, a sliding door closed and a mild footshock of either 0 mA, 1.0 mA or 1.25 mA for 1 s was delivered. The 1.0 mA footshock was selected based on previous studies in our laboratory, 1.25 mA was a mild increase while the 0 mA was used as a control. Individual rats were exposed to only one level of footshock. The rat was then removed from the chamber and tested again after 5 min. This procedure was repeated until the animal spent 5 min in the lighted chamber two consecutive times with a maximum of 5 trials. The number of trials to reach criterion was recorded. One day following the acquisition trial, the animals were administered either DU-14 (30 mg/kg, IP) or corn oil (vehicle, 1 ml/kg, IP) for 6 consecutive days. Three hours after the final treatment, the animals were again placed in the lighted compartment and the crossover latency recorded. If an animal did not enter the dark compartment within 10 min, it was removed from the apparatus, and the latency recorded as 10 min.