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Nandrolone-induced aggressive behavior is associated with alterations in extracellular glutamate homeostasis in mice

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ABSTRACT

Nandrolone decanoate (ND), an anabolic androgenic steroid (AAS), induces an aggressive phenotype by mechanisms involving glutamate-induced N-methyl-D-aspartate receptor (NMDAr) hyperexcitability. The astrocytic glutamate transporters remove excessive glutamate surrounding the synapse. However, the impact of supraphysiological doses of ND on glutamate transporters activity remains elusive. We investigated whether ND-induced aggressive behavior is interconnected with GLT-1 activity, glutamate levels and abnormal NMDAr responses. Two-month-old untreated male mice (CF1, n = 20) were tested for baseline aggressive behavior in the resident–intruder test. Another group of mice (n = 188) was injected with ND (15 mg/kg) or vehicle for 4, 11 and 19 days (short-, mid- and long-term endpoints, respectively) and was evaluated in the resident–intruder test. Each endpoint was assessed for GLT-1 expression and glutamate uptake activity in the frontoparietal cortex and hippocampal tissues. Only the long-term ND endpoint significantly decreased the latency to first attack and increased the number of attacks, which was associated with decreased GLT-1 expression and glutamate uptake activity in both brain areas. These alterations may affect extracellular glutamate levels and receptor excitability. Resident males were assessed for hippocampal glutamate levels via microdialysis both prior to, and following, the introduction of intruders. Long-term ND mice displayed significant increases in the microdialysate glutamate levels only after exposure to intruders. A single intraperitoneal dose of the NMDAr antagonists, memantine or MK-801, shortly before the intruder test decreased aggressive behavior. In summary, long-term ND-induced aggressive behavior is associated with decreased extracellular glutamate clearance and NMDAr hyperexcitability, emphasizing the role of this receptor in mediating aggression mechanisms.

Introduction

Anabolic androgenic steroids (AAS), such as nandrolone decanoate (ND), are synthetic derivatives of testosterone that were developed to improve anabolic functions with fewer androgenic effects (Jones and Lopez, 2006; Shahidi, 2001). However, humans and rodents submitted to high AAS dose regimens may display exaggerated emotional reactivity and aggressive behavior, which ultimately is associated with glutamatergic hyperexcitability in brain areas such as the hypothalamus, cortex, and hippocampus (Breuer et al., 2001; Carrillo et al., 2009, 2011a; Diano et al., 1997; Kanayama et al., 2010; Le Greves et al., 1997; McGinnis, 2004; Ricci et al., 2007; Robinson et al., 2012; Talih et al., 2007).

The mechanism underlying the AAS-induced aggressive phenotype is dynamic and not restricted to proteins of the synaptic milieu. For instance, select hypothalamic neurons express dramatic increases in phosphate-activated glutaminase, the rate-limiting enzyme in the synthesis of glutamate, in aggressive, adolescent, AAS-treated, male Syrian hamsters (Fischer et al., 2007). Steroids also increase the rate of glutamate and aspartate release, thus increasing the binding probability of glutamate to NMDA or AMPA receptors (Brann and Mahesh, 1995; Ventriglia and Di Maio, 2013). Actually, AAS-induced aggression is mechanistically associated with glutamatergic hyperexcitability. This concept has been supported by studies on genetically modified animals and pharmacological studies addressing glutamate fast-acting ionotropic receptors, i.e. kainate (KAr), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAr), and N-methyl-D-aspartate (NMDAr) (Fischer et al., 2007). To date, studies on knockout mice lacking the AMPAr 1 subunit (GluR1) show reduced aggressive behavior (Vekovischeva et al., 2004), whereas AAS-treated aggressive hamsters

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display a significant increase in the number and density of GluR1-expressing hypothalamic neurons compared to non-aggressive, vehicle-treated controls (Fischer et al., 2007). Moreover, hypothalamic administration of a KAr agonist or L-glutamate stimulates aggressive attacks in rats and cats respectively (Brody et al., 1969; Haller et al., 1998). Although pharmacological antagonism of NMDAr may cause non-specific behavioral effects due to sedation, this receptor has multiple regulatory binding sites that may serve as anti-aggressive targets (Bortolato et al., 2012; Umukoro et al., 2013). Accordingly, the administration of memantine (MEM), a low-affinity uncompetitive NMDAr antagonist, decreases aggression induced by social isolation or morphine withdrawal in rodents (Belozertseva et al., 1999; Sukhotina and Bespalov, 2000). Moreover, knockout mice for monoamino oxidase 'A' exhibited pathological aggressive behavior mediated by the higher expression of NR2A and NR2B subunits of NMDAr in the prefrontal cortex (Bortolato et al., 2012). Remarkably, systemic administration of selective NR2A and NR2B antagonists as well as dizocilpine (MK-801) an uncompetitive high-affinity NMDAr antagonist, countered the enhanced aggression (Bortolato et al., 2012). Collectively, these studies highlight a positive correlation between heightened aggression and increased glutamatergic tonus in a range of animal models.

Because there are no extracellular enzymes that can degrade glutamate, the maintenance of physiological concentrations requires glutamate transporters activity present in both astrocytes and neurons. The high-affinity glutamate transporter 1 (GLT-1) is predominantly expressed in astrocytes and is responsible for more than 90% of glutamate clearance from the synaptic cleft (Lehre and Danbolt, 1998). Further, GLT-1 is highest expressed in the hippocampus and neocortical areas (Ullensvang et al., 1997). In contrast, glutamate transporter 3 (EAAC1) is widely distributed in neurons and even GLT-1 can be found at lower levels in neurons, particularly in axon terminals. Despite neuronal EAAC1 being quantitatively lower when compared to astrocytes, its functional role cannot be neglected. Notably, it can be assumed that astrocytes (via GLT-1) are the main regulators of extracellular glutamate levels (Danbolt, 2001). Although astrocytic glutamate uptake is recognized as an important mechanism to avoid excessive glutamate levels associated with prolonged receptor activation, the impact of supraphysiological doses of ND on glutamate transporters activity remains elusive.

Here, we investigate whether ND-induced aggression is mechanistically interconnected with GLT-1 activity, glutamate levels, and NMDAr response in the brains of male, gonad-intact CF1 mice.

Material and methods

Animals

Two-month old CF1 male mice (total n = 208) weighting 32–38 g were housed in standard polycarbonate cages (cm: 28 × 17.8 × 12.7), and kept in a temperature-controlled room (22 ± 1 °C) with a 12 h light/12 h dark cycle (light on at 7 a.m.). The animals were permitted free access to food and water. To avoid social isolation, both resident and intruder mice were housed at four per cage (Leasure and Decker, 2009). All experimental procedures were performed according to the *NIH Guide for Care and Use of Laboratory Animals* and the *Brazilian Society for Neuroscience and Behavior* (SBNeC). Recommendations for animal

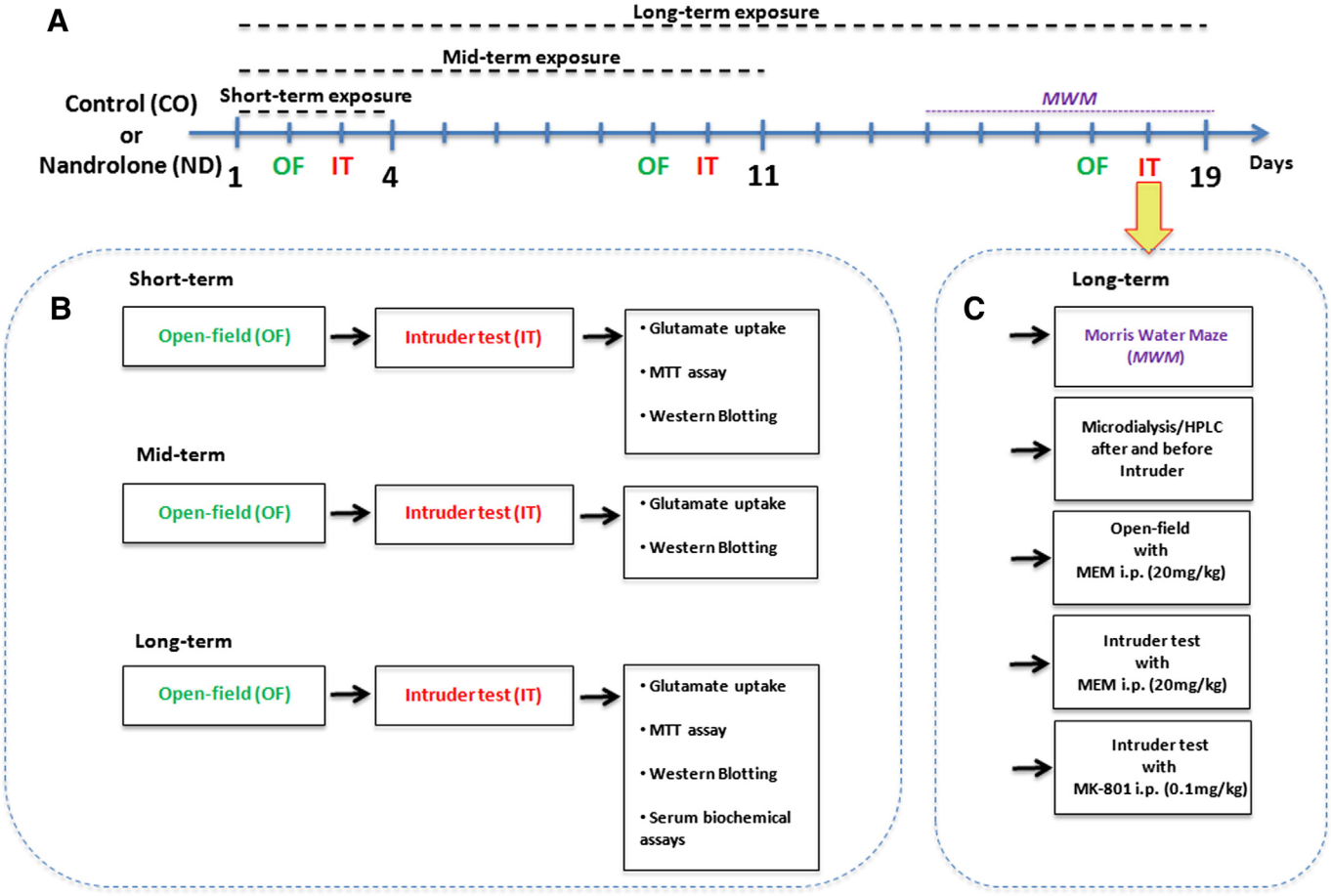

Fig. 1. Schematic experimental design. Behavioral and neurochemical endpoints in control (CO) and nandrolone (ND) groups: (A) The timeline showing each endpoint (short-, mid- and long-term exposure); (B) Flowchart representing behavioral and biochemical outcomes for each endpoint; (C) Boxes with arrows representing five independent experiments conducted after long-term exposure. Abbreviations: high-performance liquid chromatography (HPLC), memantine (MEM), dizocilpine (MK-801), intraperitoneal (i.p.).