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Neuropharmacology 49 (2005) 573–586
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

# Short-term steroid treatment increases δ $GABA_A$ receptor subunit expression in rat CA1 hippocampus: Pharmacological and behavioral effects

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Received 9 September 2004; received in revised form 13 April 2005; accepted 14 April 2005

**Abstract**

In this study, 48 h administration of 3α-OH-5β-pregnan-20-one (3α,5β-THP) or 17β-estradiol ($E_2$)+progesterone (P) to female rats increased expression of the δ subunit of the $GABA_A$ receptor (GABAR) in CA1 hippocampus. Coexpression of α4 and δ subunits was suggested by an increased response of isolated pyramidal cells to the GABA agonist 4,5,6,7- tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP), following 48 h steroid treatment, and nearly complete blockade by 300 μM lanthanum ($La^{3+}$). Because α4βδ GABAR are extrasynaptic, we also recorded pharmacologically isolated GABAergic holding current from CA1 hippocampal pyramidal cells in the slice. The $La^{3+}$-sensitive THIP current, representative of current gated by α4βδ GABAR, was measurable only following 48 h steroid treatment. In contrast, the bicuculline-sensitive current was not altered by steroid treatment, assessed with or without 200 nM gabazine to block synaptic current. However, 48 h steroid treatment resulted in a tonic current insensitive to the benzodiazepine agonists lorazepam (10 μM) and zolpidem (100 nM). These results suggest that 48 h steroid treatment increases expression of α4βδ GABAR which replace the ambient receptor population. Increased anxiolytic effects of THIP were also observed following 48 h steroid treatment. The findings from the present study may be relevant for alterations in mood and benzodiazepine sensitivity reported across the menstrual cycle.


*Keywords:* Neurosteroid; δ subunit; $GABA_A$ receptor; THIP; CA1 hippocampus; Tonic current

## 1. Introduction

The GABA-modulatory steroid 3α-OH-5α-pregnan-20-one (3α,5α-THP) or allopregnanolone and its active isomer, pregnanolone (3α,5β-THP), are metabolites of the steroid progesterone (P). Unlike most steroids, the THP isomers are potent positive modulators of the $GABA_A$ receptor (GABAR) when acutely applied (Belelli et al., 2002; Brown et al., 2002; Majewska et al., 1986; Wohlfarth et al., 2002), an effect which enhances tonic GABAergic current in areas such as dentate gyrus (Stell et al., 2003). As expected for GABA-modulatory compounds, such as benzodiazepines (BDZ) and barbiturates, these steroids possess dose-dependent anxiolytic and anticonvulsant properties (Bitran et al., 1999; Frye, 1995). However, prolonged exposure to these steroids across a period of 48–72 h, to

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0028-3908/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2005.04.026

mimic physiological fluctuations, produces alterations in GABAR subunit composition as well as reduced levels of inhibition. Increases in hippocampal expression of the GABAR α4 subunit are observed following 48 h 3α,5β-THP administration to female rats in association with increases in anxiety, assessed using two animal measures: the elevated plus maze (Gulinello et al., 2001) and the acoustic startle paradigm (Gulinello and Smith, 2003). As expected for an increase in α4-containing GABAR (Wafford et al., 1996; Wisden et al., 1991), hippocampal pyramidal cells are insensitive to BDZ modulation of GABA-gated current following 48 h steroid exposure, assessed using whole cell patch clamp techniques (Gulinello et al., 2001). A similar insensitivity to the anxiolytic effects of the BDZ lorazepam (LZM) is also observed at this time (Gulinello et al., 2001).

The α4 subunit can coexpress with either γ2 or δ subunits (Sur et al., 1999), which both produce receptors insensitive to BDZ-modulation (Brown et al., 2002; Wisden et al., 1991). However, the other pharmacological characteristics, biophysical properties, and sub-cellular localization of α4βγ2 and α4βδ GABAR are distinct (Bianchi et al., 2002; Brown et al., 2002; Peng et al., 2002). For instance, the GABA partial agonist 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP) or gaboxadol generates a current greater than maximum concentrations of GABA only at α4[6]βδ GABAR (Brown et al., 2002) but not at other αβγ2 subtypes (Ebert et al., 1997). In addition, the trivalent cation lanthanum ($La^{3+}$) is a potent inhibitor of current gated by α4[α6]βδ GABAR (Brown et al., 2002; Saxena et al., 1997; Zhu et al., 1998), while it produces minimal inhibition of α4[6]βγ2 GABAR, and potentiates α1βγ2 and α1βδ GABAR (Saxena et al., 1997; Zhu et al., 1998).

In contrast to γ2-containing GABAR, δ-containing GABAR are localized exclusively to extrasynaptic sites (Nusser et al., 1998; Wei et al., 2003), where they produce a tonic current (Rossi and Hamann, 1998; Wei et al., 2003). In cerebellar and dentate gyrus granule cells this tonic current is believed to act as a resistive shunt to limit excitability of the neuron (Brickley et al., 2001). Recent studies have reported that for neurosteroids and sedative drugs such as anesthetics, BDZs and ethanol, effects on tonic current are greater in magnitude than observed at the synapse (Bai et al., 2000; Belelli and Herd, 2003; Stell et al., 2003; Wei et al., 2004; Yeung et al., 2003). Therefore, the tonic current may have important implications for the behavioral effects of these GABA-modulatory compounds, as well as for establishing cellular mechanisms.

Although our previous work suggests that α4βγ2 GABAR expression increases following 48 h steroid administration (Hsu et al., 2003), levels of δ subunit expression have yet to be tested. This is especially warranted because we have recently reported that a steroid withdrawal paradigm which increases hippocampal expression of the α4 GABAR subunit results in α4βδ coexpression (Sundstrom-Poromaa et al., 2002). Therefore, in the present study, both molecular and pharmacological techniques were employed to determine both δ subunit expression and α4βδ GABAR coexpression following 48 h treatment of female rats with 3α,5β-THP. In addition, because the steroid 17β-estradiol ($E_2$) can act in a permissive fashion to produce the in vitro BDZ insensitivity associated with steroid withdrawal (Costa et al., 1995), similar studies were carried out to quantify α4 and δ subunit expression following combined administration of $E_2$+P for 48 h. Finally, in order to demonstrate a physiologically relevant outcome for these pharmacological changes related to α4βδ GABAR expression, the anxiolytic effects of THIP were tested using the elevated plus maze following 48 h steroid exposure. The results of the present study may be important in furthering our understanding of the homeostatic mechanisms resulting from sustained exposure to endogenous GABA modulators such as 3α,5α[β]-THP.

## 2. Materials and methods

### 2.1. Experimental animals

Adult, female Long Evans rats (Charles River, 140–200 g), housed in groups of three, were used for all protocols. Animals were maintained under controlled conditions of light (14 h light:10 h dark) and temperature (21 °C) with free access to food and water. Animals were used during the light phase of the circadian cycle, 1 h following steroid or vehicle injection. Control rats were tested on the day of diestrus−1, a low hormone stage of the cycle, verified by microscopic examination of the vaginal lavage, a routine procedure. All protocols were conducted under guidelines established by the Institutional Animal Care and Use Committee.

### 2.2. Steroid administration paradigm

Animals were injected intraperitoneally with either (i) 3α,5β-THP (3α-OH-5β-pregnan-20-one, 10 mg/kg), (ii) P (progesterone, 25 mg/kg), (iii) $E_2$ (17β-estradiol, 8 μg/kg)+P (progesterone, 25 mg/kg) or (iv) oil vehicle. Injections were administered daily for 3 days beginning on diestrus−1, and the animals sacrificed 1 h following the final injection. These steroid administration paradigms have been shown to result in physiological levels of circulating steroids (Moran and Smith, 1998). Intact, non-ovariectomized rats were used for the study because ovariectomy has been shown to alter CNS neurosteroid metabolism following injection of exogenous P (Corpechot et al., 1993).