Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 89 (2008) 171–178
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

# Involvement of neurosteroids in the anxiolytic-like effects of AC-5216 in mice

Atsuko Kita *, Kiyoshi Furukawa

*Discovery Pharmacology II, Pharmacology Research Laboratories, Dainippon Sumitomo Pharma Co., Ltd., Enoki 33-94, Suita, Osaka 564-0053, Japan*

Received 13 September 2007; received in revised form 26 November 2007; accepted 5 December 2007
Available online 14 December 2007

**Abstract**

AC-5216, a ligand for the translocator protein (18 kDa) (TSPO), previously called the peripheral benzodiazepine receptor (PBR), produces anxiolytic-like effects mediated by TSPO in animal models of anxiety. Since stimulation of TSPO is considered to promote the synthesis of neurosteroids, we investigated the possible role of endogenous neurosteroids that positively act on the $GABA_A$ receptor in the anxiolytic-like effects of AC-5216. In our experiments, the effects of trilostane and finasteride, two inhibitors of steroidogenic enzymes, and picrotoxin, a $GABA_A$ receptor-gated $Cl^-$ channel blocker, on the anxiolytic-like effects of AC-5216 were examined in the social interaction test in mice. Also, the anxiolytic-like effects of allopregnanolone and progesterone were examined. The anxiolytic-like effects of AC-5216 (0.1 mg/kg, p.o.) were inhibited by trilostane (10–30 mg/kg, s.c.), finasteride (10–30 mg/kg, s.c.), and picrotoxin (0.03–0.3 mg/kg, s.c.), while those of diazepam (0.1 mg/kg, p.o.) were inhibited by picrotoxin only. The anxiolytic-like effects of progesterone (1–3 mg/kg, s.c.) were inhibited by finasteride (3–30 mg/kg) and picrotoxin (0.1–0.3 mg/kg), although those of allopregnanolone (10 mg/kg, s.c.) were inhibited by picrotoxin only. These results demonstrate that the anxiolytic-like effects of AC-5216 are due to newly synthesized neurosteroids that enhance $GABA_A$ receptor function.


*Keywords:* AC-5216; Anxiolytic effect; Translocator protein (18 kDa) (TSPO); Peripheral benzodiazepine receptor (PBR); Mitochondrial benzodiazepine receptor (MBR); Neurosteroid; Trilostane; Finasteride; Picrotoxin; Social interaction; Mice

## 1. Introduction

The translocator protein (18 kDa) (TSPO) (Papadopoulos et al., 2006), previously called the peripheral benzodiazepine receptor (PBR) or the mitochondrial benzodiazepine receptor (MBR), was initially identified as a peripheral binding site for diazepam, and later distinguished functionally and structurally from the central benzodiazepine receptor (CBR) (Anholt et al., 1984; Beurdeley-Thomas et al., 2000; Casellas et al., 2002). CBR is located on the extracellular domain of $GABA_A$ receptor, and its agonists, such as benzodiazepine anxiolytics, are known to allosterically potentiate the inhibitory action of GABA (Mohler et al., 2002). In contrast, TSPO is located mainly in the outer mitochondrial membrane in peripheral tissues and central nervous system (CNS), and is not linked to the $GABA_A$ receptor (Anholt et al., 1984; Anholt et al., 1986; Basile and Skolnick, 1986). In the CNS, TSPO is mainly located in glial cells (Gallager et al., 1981; Schoemaker et al., 1982) and in neurons (Anholt et al., 1984; Doble et al., 1987). Although the function of TSPO in the CNS remains to be fully disclosed, several lines of evidence have been provided for the involvement of TSPO in synthesis of neurosteroids (Krueger and Papadopoulos, 1990; Papadopoulos et al., 1997). Stimulation of TSPO by appropriate ligands increases the level of neurosteroids (Korneyev et al., 1993; Serra et al., 1999), and it is suggested that this increase is due to TSPO's potential to facilitate the transport of cholesterol from the outer to the inner mitochondrial membrane (Papadopoulos et al., 1997). This transport of cholesterol is known as the rate-limiting step in the synthesis of neurosteroids (Stocco, 2001). In the mitochondria, cholesterol is converted to pregnenolone by $P450_{SCC}$ located in the inner mitochondrial membrane. Pregnenolone moves then to the cytosol where it is processed by several enzymes in a cascade of neurosteroidogenesis. For example, the microsomal enzymes 3β-hydroxysteroid dehydrogenase (3β-HSD) converts pregnenolone to progesterone, which is further metabolized to

* Corresponding author. Tel.: +81 6 6337 5920; fax: +81 6 6337 5109.
*E-mail address:* atsuko-kita@ds-pharma.co.jp (A. Kita).

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2007.12.006

allopregnanolone by the microsomal enzymes 5α-reductase and 3α-hydroxysteroid oxidoreductase.

Neurosteroids exert non-genomic effects; they alter neuronal excitability by modulating the activity of several neurotransmitter receptors such as GABA, glutamate and acetylcholine receptors and thus can influence emotion, memory/learning and stress–response (Dubrovsky, 2005; Mellon and Griffin, 2002; Strous et al., 2006). Progesterone-reduced metabolites such as allopregnanolone are known to positively modulate $GABA_A$ receptor function *in vitro* (Gee et al., 1988) and to produce anxiolytic-like effects in several animal models of anxiety (Bitran et al., 1991; Gomez et al., 2002; Picazo and Fernandez-Guasti, 1995; Rodgers and Johnson, 1998; Wieland et al., 1995). These findings suggest that TSPO ligands with anxiolytic effects could exert their action via newly synthesized neurosteroids.

Recently, we have demonstrated that *N*-benzyl-*N*-ethyl-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9*H*-purin-9-yl)acetamide (AC-5216), a novel TSPO ligand synthesized in our laboratories, exhibits high and selective affinity for TSPO derived from rats and human, and that oral administration of AC-5216 produces anxiolytic-like effects in the Vogel-type conflict test in rats, the light/dark box and social interaction tests in mice, and antidepressant-like effects in the forced swimming test in rats (Kita et al., 2004). We have also demonstrated that the anxiolytic-like effects of AC-5216, unlike those of diazepam, are blocked by PK11195, a potent TSPO ligand with presumed *in vivo* antagonistic activity for TSPO, but not affected by flumazenil, a CBR antagonist (Kita et al., 2004). These findings indicate that the anxiolytic-like effects of AC-5216 are mediated by the TSPO.

In the present study, we investigated the possible role of endogenous neurosteroids that positively act on the $GABA_A$ receptor, such as allopregnanolone, in the mechanism underlying the anxiolytic-like effects of AC-5216. In our experiments, the effects of trilostane, a 3β-HSD inhibitor (Potts et al., 1978) and finasteride, a 5α-reductase inhibitor (Rittmaster, 1994; Rittmaster, 1997), and picrotoxin, a $GABA_A$ receptor-gated $Cl^-$ channel blocker, on the anxiolytic-like effects of AC-5216 were evaluated in the social interaction test in mice. To confirm that the action "if any" of these inhibitors on the anxiolytic-like effects of AC-5216 is specific to the mechanism of the action of AC-5216, we evaluated the effects of these inhibitors on the anxiolytic effects of diazepam, which is known to act via CBR, not TSPO (Kita et al., 2004). In addition, although neurosteroids have been reported to exert anxiolytic-like effects in several animal models of anxiety (Bitran et al., 1991; Brot et al., 1997; Picazo and Fernandez-Guasti, 1995; Rodgers and Johnson, 1998), little information on the anxiolytic-like effects of neurosteroids in the social interaction test has been reported (Frye and Rhodes, 2006). Therefore, we evaluated, in this study, the anxiolytic-like effects of allopregnanolone and its precursor progesterone in the social interaction test in mice.

## 2. Materials and methods

### 2.1. Animals

Male ddY mice, aged 5 weeks and weighing 22 to 32 g at the time of experiments, were obtained from Japan SLC Inc. (Shizuoka, Japan). In total, 960 mice were used for 17 experiments in this study. Animals were maintained for at least 5 days before experiments in a temperature- and humidity-controlled animal room under a 12:12 h light/dark cycle (light on 06:00 to 18:00) with free access to food and water. They were housed in groups of 5, with the same mates throughout the acclimation and testing period. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Dainippon Sumitomo Pharma Co., Ltd.

### 2.2. Compounds

AC-5216 was synthesized in our Chemistry Research Laboratories. Allopregnanolone (5α-pregnan-3α-ol-20-one) was purchased from Sigma-Aldrich (SIGMA®, St. Louis, MO, USA), diazepam from Wako Pure Chemical Industries Ltd. (Osaka, Japan), progesterone and picrotoxin from Nacalai Tesque, Inc. (Kyoto, Japan). Trilostane and finasteride were prepared from commercially available formulations in our laboratories. AC-5216 and diazepam were suspended in 0.5% tragacanth gum

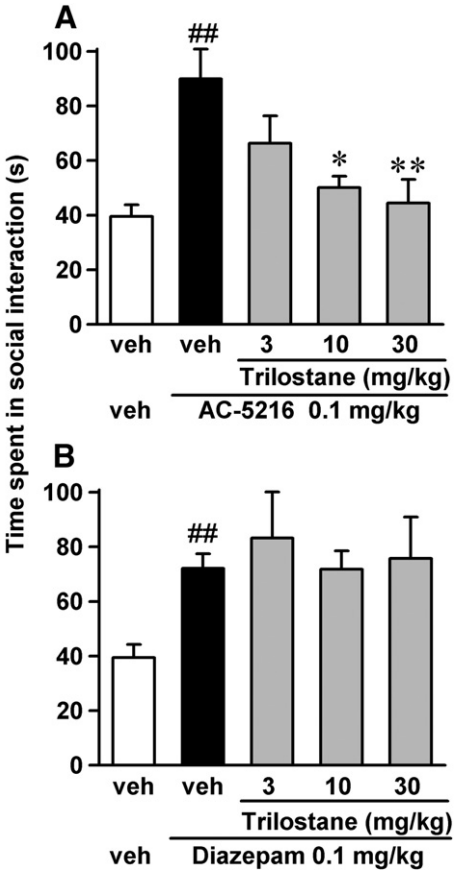


Fig. 1. Effects of trilostane on the anxiolytic-like effects of AC-5216 (A) and diazepam (B) in the social interaction test in mice. Each column represents the mean±S.E.M. of the time spent in social interaction during a 15-min period. $n=5$ pairs. AC-5216 (0.1 mg/kg), diazepam (0.1 mg/kg) or the vehicle (0.5% tragacanth gum solution) was administered orally 1 h before testing. Trilostane or the vehicle (saline containing 0.4% Tween 80) was administered s.c. 1 h before testing. ## $P<0.01$, significantly different from the respective vehicle control group (Student's *t*-test). * $P<0.05$, ** $P<0.01$, significantly different from AC-5216 alone-treated group (Dunnett's test).