Available online at www.sciencedirect.com
ScienceDirect
Neurochemistry International 52 (2008) 1212–1219
NEUROCHEMISTRY International
www.elsevier.com/locate/neuint

Sex-differences and stress: Effects on regional high and low affinity [^{3}H]GABA binding

K.J. Skilbeck [a,b,*], T. Hinton [a,b], G.A.R. Johnston [a,b]

[a] *Department of Pharmacology, Blackburn D06, The University of Sydney, NSW 2006, Australia*
[b] *Schizophrenia Research Institute, Australia*

Received 17 August 2007; received in revised form 17 December 2007; accepted 2 January 2008
Available online 6 January 2008

Abstract

Sex-differences are observed in the GABAergic neurotransmitter system both at rest and following acute stress, yet the brain regions and functional implications of these differences are unknown. We examined sex-differences in the number of low- and high-affinity [^{3}H]GABA binding sites in various brain regions of male and female mice and the effect of stress on such sex-differences. Male ($n = 6$) and female ($n = 6$) QS mice were exposed to a brief swim stress (3 min at 32 ± 1 °C) either individually or with cage-mates whilst control males ($n = 6$) and females ($n = 6$) remained undisturbed in the home cage. Using quantitative receptor autoradiography, sections of mouse brain were labelled with either 30 or 1000 nM [^{3}H]GABA to label high or low affinity binding sites, respectively. Results indicated that males had more low affinity [^{3}H]GABA binding sites in various forebrain cortical regions but less high affinity binding sites in many of these regions compared with females. Forced swim stress-induced rapid changes in forebrain GABA binding sites in females and group stressed males, suggesting a mechanism for rapid GABAergic adaptations. However the number of functional binding sites for GABA in certain forebrain regions was altered by stress in opposite directions in males and females, such that baseline sex-differences were removed following stress. These results exemplify sex-differences in brain chemical function and stress responses, and are of potential importance for understanding sex-differences in response to GABAergic compounds and disorders with sex and stress as predisposing factors.

Keywords: Stress; Sex-differences; GABA-A receptor; Autoradiography; GABA

GABA$_A$ receptors for the inhibitory neurotransmitter GABA are comprised of 5 protein subunits surrounding a chloride ion channel. The orthosteric (agonist) binding site has been extensively studied using radiolabelled agonists such as [^{3}H]GABA and [^{3}H]muscimol and antagonists such as [^{3}H]bicuculline and [^{3}H]SR 95531. Analysis of Scatchard plots from such studies has lead to a general consensus that there exists both high affinity (nM) and low affinity (nM–μM) binding sites. Whether these different binding site populations represent different conformations of the same binding site, or distinct sites on the same or different macromolecular complexes is unknown (Harris and Allan, 1985; Cash and Subbarao, 1987; Edgar and Schwartz, 1992; Smith and Olsen, 1994; Maksay, 1996; Baur and Sigel, 2003; Yeung et al., 2003). However, electrophysiological studies on cerebellar neuronal patches (Maconochie et al., 1994) and recombinant receptors (Baur and Sigel, 2003) as well as studies of chloride uptake into brain vesicle preparations (Harris and Allan, 1985) all show that μM concentrations of GABA are required for channel opening, suggesting that the low affinity GABA binding site represents the functional site.

Several lines of evidence indicate a sexual differentiation of the adult GABAergic nervous system. The majority of studies suggest that males are more sensitive to the behavioural effects of compounds that act on GABA$_A$ receptors such as ethanol (high doses) (Crippens et al., 1999; Tayyabkhan et al., 2002; Webb et al., 2002), diazepam (low doses) (Fernandez-Guasti and Picazo, 1997; Wilson et al., 2004), allopregnanalone (Fernandez-Guasti and Picazo, 1999; Gulinello and Smith, 2003), pentylenetetrazol (PTZ) (Kokka et al., 1992), picrotoxin (i.v) (Peričić and Bujas, 1997) and bicuculline (i.v) (Manev

* Corresponding author at: Department of Pharmacology, Blackburn D06, The University of Sydney, NSW 2006, Australia. Fax: +61 2 9351 3868.
E-mail address: kskilbeck@med.usyd.edu.au (K.J. Skilbeck).

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doi:10.1016/j.neuint.2008.01.001

et al., 1987; Wilson, 1992; Guillet and Dunham, 1995; Bujas et al., 1997; Peričić and Bujas, 1997; Peričić et al., 1999). Drug administration route (Peričić et al., 1986), species differences (Manev et al., 1987; Peričić and Bujas, 1997) and sex-differences in metabolism of some of these drugs (Peričić and Bujas, 1997) raise the possibility that sex-differences arise from variations in drug pharmacokinetics. Furthermore, the influence of factors such as drug dose (Wilson et al., 2004) makes it difficult to say that sex-differences in response to GABAergic compounds are robust. However, studies have shown that, at least for ethanol, male neurons are indeed more sensitive to allosteric site enhancement of GABAergic currents (Cha et al., 2006) a finding that is consistent with underlying sex-differences in the GABAergic system.

Sex-differences in the sensitivity to compounds that act on $GABA_A$ receptors may arise from sex-differences in $GABA_A$ receptor expression or sensitivity, yet the literature is inconsistent on this topic. There is clear evidence for sexual dimorphism of [^{3}H]muscimol binding at high affinity $GABA_A$ receptor binding sites (Juptner and Hiemke, 1990; Kokka et al., 1992) and [^{3}H]flunitrazepam binding (Shephard et al., 1982) at allosteric benzodiazepine sites in various brain regions. However, no differences are observed in cortical and cerebellar [^{3}H]bicuculline binding at the low affinity $GABA_A$ receptor site (Wilson, 1992; Wilson and Biscardi, 1992; Bujas et al., 1997). Studies examining the functional state of the receptor by measuring [^{35}S]TBPS binding have found no sex-differences in receptor function but females do show a lower GABA IC_{50} than males for displacement of [^{3}H]bicuculline binding (Kokka et al., 1992). Thus, an understanding of whether functional low affinity GABA binding sites vary between sexes and the regions involved in such sex-differences is highly warranted.

Stress-induced changes in $GABA_A$ receptor binding at the orthosteric site are sexually dimorphic. Studies measuring [^{3}H]GABA binding show a rapid increase in the availability of low affinity binding sites (B_{max}) and a smaller decrease in the high affinity binding sites following acute swim stress in females (Skerritt et al., 1981; Akinci and Johnston, 1993; Wilson and Biscardi, 1994; Akinci and Johnston, 1997). In contrast, male rats and mice exposed to acute swim stress showed no changes in [^{3}H]GABA binding (Skerritt et al., 1981; Motohashi et al., 1993). Yet, studies of other stress paradigms have shown large reductions in forebrain low affinity [^{3}H]GABA binding (Biggio et al., 1981; Concas et al., 1985; Cuadra and Molina, 1993). Decreased low affinity [^{3}H]GABA binding suggests a reduction in the number of functional $GABA_A$ receptor sites, consistent with the observed changes in non-functional sites labelled by [^{35}S]TBPS following various stressors (Havoundjian et al., 1986; Concas et al., 1987, 1988, 1993). Thus stress appears to induce an increase in functional binding sites in females but a decrease in males, however it is unknown if these effects are regionally specific.

To better understand sex-differences in $GABA_A$ receptor expression and sensitivity, in the current investigation we mapped both high and low affinity [^{3}H]GABA binding in several brain regions of male and female mice. To determine the brain regions where $GABA_A$ receptor density is altered following an acute stress, we also examined high and low affinity [^{3}H]GABA binding sites in animals following a 3 min swim stress. Additionally, as it has been suggested that the presence of conspecifics may influence stress responses differently in males (Cuadra and Molina, 1993; Taylor et al., 2000; Troisi, 2001), we examined animals that were stressed either individually or with their cage-mates.

1. Materials

[^{3}H]GABA (87 Ci/mmol), Kodak Biomax MS film, autoradiography cassettes and tritium microscale standards (2.0–110.0 nCi/mg and 0.1–15.4 nCi/mg) were purchased from G.E Healthcare (Castle Hill, NSW, Australia). Hydrochloric acid for pH adjustments was purchased from APS Finechem (Seven Hills, Australia). Tissue-Tek-OCT embedding compound was purchased from Sakura Finetechnical (Tokyo, Japan). Phenisol developer and Hypam Rapid Fixer were obtained from Ilford (Mt Waverley, NSW, Australia). All other chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2. Methods

2.1. Subjects

Eighteen female and eighteen male Quakenbush Swiss (QS) albino mice aged 8 weeks (Laboratory Animal Services, Perth, WA) were housed in groups of three upon arrival at the animal house. All mice were housed under a 12 h/12 h light/dark cycle with constant temperature (21 °C) and permitted food and water *ad libitum*. Animals were allowed to habituate to the environment for 1 week and were then handled by experimenters for an additional week prior to acute stress protocols. The experiment protocols were approved by the Animal Ethics Committee of The University of Sydney.

2.2. Acute swim stress

On the day of experimentation, mice were habituated to the experimental room for 1 h prior to procedures. Cages of mice were randomly assigned to either individual or group conditions. Within cages assigned to the individual condition, mice were randomly assigned to either control or acute stress conditions. As a result there were six experimental groups with $n = 6$ subjects per group; male control, male individual stress, male group stress, female control, female individual stress, female group stress. Mice assigned to a stress condition were swum between 11 a.m. and 1 p.m. either individually or in groups of three for 3 min in water at 32 ± 1 °C at 10 cm depth in a 39 cm × 20 cm × 15 cm Perspex container. Control mice remained in their home cage for the same period. Mice were swum on 2 consecutive days with $n = 3$ per group per day.

2.3. Tissue acquisition and preparation

Following the 3 min swim stress, animals were sacrificed by cervical dislocation and immediately decapitated. Brains were removed from the cranium over ice then immediately immersed in liquid isopentane on dry ice for 15 s to ensure rapid freezing. Tissue was stored at −70 °C until sectioning. Coronal sections were cut at 12 μm thickness in a cryostat (Damon/IEC Division, Nedham Heights, MA, USA) maintained at −14 °C then thaw-mounted onto slides pre-treated with 2% silane in acetone. Sections were cut from three blocks at levels of bregma; 1.1 to 0.62 mm, −1.0 to 1.34 mm, −1.7 to 2.18 mm (Paxinos and Franklin, 2001). Three sections were mounted per slide and slides were stored for a maximum of 12 days at −70 °C prior to receptor binding assays.