Available online at www.sciencedirect.com
SCIENCE DIRECT®
Life Sciences 79 (2006) 342–350
Life Sciences
www.elsevier.com/locate/lifescie

Adenohypophyseal and hypothalamic GABA B receptor subunits are downregulated by estradiol in adult female rats

Estela B. Rey-Roldán [a,d], María S. Bianchi [a], Bernhard Bettler [b], Damasia Becu-Villalobos [a], Victoria A. Lux-Lantos [a,*], Carlos Libertun [a,c]

[a] Instituto de Biología y Medicina Experimental-CONICET, Buenos Aires, Argentina
[b] Department of Clinical-Biological Sciences, University of Basel, Basel, Switzerland
[c] Faculty of Medicine, Universidad de Buenos Aires, Buenos Aires, Argentina
[d] Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

Received 14 October 2005; accepted 9 January 2006

Abstract

Gamma-aminobutyric acid (GABA) participates in neuroendocrine regulation. Since steroid hormones have been shown to modulate the GABAergic system, here we evaluated the effect of chronic in vivo estradiol administration on GABA B receptor ($GABA_BR$) expression.

$GABA_{B1}$ and $GABA_{B2}$ subunits were analyzed by Western Blot and RT-PCR, in hypothalami and anterior pituitaries of adult female rats: a) treated for 1 week with estradiol–valerate (a single dose of 100 μg /kg: E1), b) implanted with a 10 mg pellet of estradiol–benzoate for 5 weeks (E5) or c) on proestrous (P), d) ovariectomized (OVX). Pituitary $GABA_BR$ levels were correlated to a biological effect: baclofen, a $GABA_BR$ agonist, action on intracellular calcium titers ($[Ca^{2+}]_i$) in pituitary cells.

E5 pituitaries showed a significant decrease in the expression of $GABA_{B1}$ and $GABA_{B2}$ mRNAs compared to P. The $GABA_{B1a}$ splice variant of $GABA_{B1}$ was always more abundant than $GABA_{B1b}$ in this tissue. Similar to the pituitary, hypothalamic $GABA_{B1}$ and $GABA_{B2}$ mRNAs decreased in E5; this was confirmed at the protein level. In the hypothalamus $GABA_{B1b}$ was the main variant expressed in P rats, and was the one significantly sensitive to estradiol-induced decrease, as determined by Western Blots. Castration did not modify $GABA_BR$ expression with regards to P in either tissue. In P pituitary cells baclofen induced a decrease in $[Ca^{2+}]_i$, in contrast this effect was lost in E5 cells.

We conclude that chronic estradiol treatment negatively regulates the expression of the $GABA_BR$ subunits in the pituitary and the hypothalamus. This effect is coupled to a loss of baclofen action on intracellular calcium in pituitary cells.

Keywords: $GABA_B$ receptors; Pituitary; Hypothalamus; Estradiol; $[Ca^2]_i$ oscillations; Female rats

Introduction

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (CNS). It also participates in the control of hypophyseal secretion, acting at the CNS and directly at the pituitary level (Demeneix et al., 1986; Donoso and Banzan, 1984; Fiszer de Plazas et al., 1982; Grandison, 1981; Libertun et al., 1979; Tuomisto and Mannisto, 1985). Pituitary GABA derives from tuberoinfundibular GABA, the intermediate pituitary lobe and local synthesis (Gamel-Didelon et al., 2002; Vincent et al., 1982).

This amino acid neurotransmitter acts on three types of receptors, $GABA_A$, $GABA_B$ and $GABA_C$ receptors ($GABA_ARs$, $GABA_BRs$ and $GABA_CRs$). $GABA_{A/C}Rs$ are ionotropic and belong to the superfamily of ligand-gated ion channels. $GABA_BRs$ are metabotropic, functionally coupled to Gi/0 proteins, specifically activated by baclofen and their "in vitro" activation inhibits PRL and gonadotropins (Lux-Lantos et al., 1992; Rey-Roldán et al., 1996), and stimulates GH secretion (Gamel-Didelon et al., 2002). In addition, they are also expressed in melanotropes, participating in the regulation of αMSH output (Purisai et al., 2005). We have previously

* Corresponding author. V. de Obligado 2490, C1428ADN Buenos Aires, Argentina. Tel.: +54 11 4783 2869; fax: +54 11 4786 2564.
E-mail address: libertun@dna.uba.ar (V.A. Lux-Lantos).

0024-3205/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2006.01.014

described that anterior pituitary $GABA_BRs$ are negatively coupled to voltage-gated calcium channels (VSCC) through Pertussis toxin sensitive G proteins (Lux-Lantos et al., 2001), similar to CNS $GABA_BRs$ (Bowery, 1999).

$GABA_BRs$ are heterodimers formed by a $GABA_{B1}$ and a $GABA_{B2}$ subunits (Bowery et al., 2002; Kaupmann et al., 1998). There are two main isoforms of $GABA_{B1}$, $GABA_{B1a}$ and $GABA_{B1b}$, generated by distinct promoters and differing in their N-terminal extracellular domain (Kaupmann et al., 1997). Previously, we reported particular pituitary $GABA_BR$ ontogenic expression patterns in both sexes in the rat (Bianchi et al., 2001), neonatal testosterone being involved in sexual differences encountered (Bianchi et al., 2004). We have also observed ontogenic sex differences in $GABA_BR$ expression in rat hypothalamus (Bianchi et al., 2005). Chronic exposure to estrogen has profound effects on the hypothalamic–pituitary unit. Among the plethora of effects described for this steroid on the pituitary, the induction of a very significant hyperplasia and hypertrophia of lactotropes, with pituitary enlargement, has been extensively characterized (De Nicola et al., 1978; Gonzalez et al., 2000). Furthermore estrogen treatment produces involution of somatotrophs and gonadotropin-producing cells (De Nicola et al., 1978). In addition, it has been shown that estrogens alter the sensitivity of adenohypophyseal cells to stimulatory and inhibitory factors, such as dopamine, TRH, angiotensin II and GABA (Apud et al., 1985; Diaz-Torga et al., 1998; Livingstone et al., 1998; Suarez et al., 2003, 2004). Moreover, ovarian steroid hormones modulate GABAergic neurotransmission in various brain areas by altering $GABA_AR$ function or subunit composition (Herbison and Fenelon, 1995; Schumacher et al., 1989; Weiland and Orchinik, 1995). Steroid hormones have also been reported to modify $GABA_BRs$ function or expression in the CNS. Binding of baclofen to neocortical, hippocampal or hypothalamic membranes varied as a function of the estrous cycle, with the lowest binding occurring at different stages of the estrous cycle depending of the area analyzed (al-Dahan et al., 1994). Furthermore, in ovariectomized rats, subcutaneous injection of progesterone alone, without estrogen priming, increased the binding of baclofen to $GABA_BRs$ in the neocortex (al-Dahan and Thalmann, 1996). With regard to estrogens, it has been reported that a short exposure to estradiol rapidly blunts the inhibitory response evoked by $GABA_BR$ stimulation in discrete populations of hypothalamic neurons by uncoupling these receptors from K^+ channels (Lagrange et al., 1996). It has also been shown that estradiol enhances the Pertussis toxin ADP-ribosylation of Gi/0 proteins in the striatum (Kelly and Wagner, 1999). In addition, in the pituitary, estradiol induces a decrease in the expression of Gi/0 proteins, essential for $GABA_BR$ function (Livingstone et al., 1998).

In this work we evaluated the effect of chronic "in vivo" estradiol exposure on $GABA_BR$ subunits expression in neuroendocrine tissues: the hypothalamus and the anterior pituitary. In correlation we determined if differences in $GABA_BR$ expression encountered at the pituitary level modified baclofen action on intracellular calcium concentration in pituitary cells.

Materials and methods

Animals

Female Sprague–Dawley rats from the Instituto de Biología y Medicina Experimental colony were used. Adult animals (200–250 g) were implanted with subcutaneous pellets of 10 mg of estradiol–benzoate for a period of five weeks (E5) or injected with a single sc dose of estradiol–valerate (100 μg/kg, Progynon Depot, Schering, Buenos Aires, Argentina, dissolved in castor oil) for studies of one week estradiol treatment (E1). Determinations were also performed in proestrous rats (P) and in ovariectomized animals, sacrificed two weeks postcastration (OVX), both groups injected with castor oil. All groups of animals were sacrificed by decapitation between 9:00 and 10:00 AM, following protocols for animal use approved by the Institutional Animal Care and Use Committee (IBYME-CONICET) and by the NIH.

Membrane preparation

Anterior pituitaries, separated mechanically from neurointermediate lobes with very fine pincers under a magnifying glass, and hypothalami (limited anteriorly by the optic chiasma, laterally by the hypothalamic fissures, posteriorly by the mammillary bodies and in depth by the subthalamic sulcus, and including the preoptic–suprachiasmatic area) from proestrous and estrogenized female rats were collected and the membrane fraction was isolated, as previously described (Bianchi et al., 2001). Briefly, tissues were homogenized in 10 volumes of ice-cold 0.32 M sucrose, containing 1 mM $MgCl_2$, 1 mM K_2HPO_4 with a glass/Teflon homogenizer. Membranes were centrifuged at 750 ×*g*, the pellet was resuspended and the centrifugation repeated. The supernatants were pooled and centrifuged at 18,000 ×*g* for 15 min. The pellet was osmotically shocked, centrifuged at 39,000 ×*g*, resuspended in 50 mM Tris–HCl, 2.5 mM Cl_2Ca, pH 7.4 (10 vol/g of original tissue). Membranes were frozen at −70 °C.

Western blot analysis

Western blot analysis of $GABA_BR$ subunits was performed as previously described (Bianchi et al., 2001, 2004). Briefly, 50 μg of proteins of hypothalamus or pituitary membrane preparations were subjected to 8% SDS-PAGE. Proteins were transferred onto nitrocellulose by standard wet electrophoretic transfer in 0.2 M phosphate buffer. Blots were blocked in NETG buffer (159 mM NaCl; 5 mM EDTA; 50 mM Tris–HCl, pH 7.4; 0.05% Triton X-100; 0.25% gelatin) overnight at 4 °C. $GABA_BR$ subunits were detected by incubating for one hour at room temperature with antibodies Ab174.1 (1:3000) (Malitschek et al., 1998) and AbC22 (1:3000) (Kaupmann et al., 1998) directed against the C-terminal epitopes of $GABA_{B(1a/b)}$ or $GABA_{B2}$ subunits, respectively. Secondary antibody was horseradish peroxidase coupled (1:3000, Santa Cruz Biotechnology, Inc., CA). Blots were washed following each antibody incubation for 50 min with NETG. To ensure comparable protein