ELSEVIER

Animal Reproduction Science 111 (2009) 235–248

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Effects of $GABA_A$ receptor modulation on the expression of *GnRH* gene and *GnRH* receptor (*GnRH-R*) gene in the hypothalamus and *GnRH-R* gene in the anterior pituitary gland of follicular-phase ewes

Magdalena Ciechanowska[a], Magdalena Łapot[a], Tadeusz Malewski[b], Krystyna Mateusiak[a], Tomasz Misztal[a,*], Franciszek Przekop[a]

[a] *The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna n. Warsaw, Poland*

[b] *Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 3, 00-679 Warsaw, Poland*

Received 10 October 2007; received in revised form 18 February 2008; accepted 14 March 2008
Available online 20 March 2008

Abstract

The effect of prolonged, intermittent infusion of $GABA_A$ receptor agonist (muscimol) or $GABA_A$ receptor antagonist (bicuculline) into the third cerebral ventricle on the expression of *GnRH* gene and *GnRH-R* gene in the hypothalamus and *GnRH-R* gene in the anterior pituitary gland was examined in follicular-phase ewes by real-time PCR. The activation or inhibition of $GABA_A$ receptors in the hypothalamus decreased or increased the expression of *GnRH* and *GnRH-R* genes and LH secretion, respectively. The present results indicate that the GABAergic system in the hypothalamus of follicular-phase ewes may suppress, via hypothalamic $GABA_A$ receptors, the expression of *GnRH* and *GnRH-R* genes in this structure. The decrease or increase of GnRH-R mRNA in the anterior pituitary gland and LH secretion in the muscimol- or bicuculline-treated ewes, respectively, is probably a consequence of parallel changes in the release of GnRH from the hypothalamus activating *GnRH-R* gene expression. It is suggested that GABA acting through the $GABA_A$ receptor

* Corresponding author. Tel.: +48 22 782 4175; fax: +48 227742038.
E-mail addresses: m.ciechanowska@ifzz.pan.pl (M. Ciechanowska), m.lapot@ifzz.pan.pl (M. Łapot), t.malewski@ighz.pan.pl (T. Malewski), t.misztal@ifzz.pan.pl (T. Misztal).

doi:10.1016/j.anireprosci.2008.03.006

mechanism on the expression of *GnRH* gene and *GnRH-R* gene in the hypothalamus may be involved in two processes: the biosynthesis of GnRH and the release of this neurohormone in the hypothalamus.

Keywords: Hypothalamus; Pituitary gland; $GABA_A$; GnRH mRNA; GnRH-R mRNA; LH

1. Introduction

A large body of evidence indicates that GABA-ergic neurons in the hypothalamus are involved in the controlling GnRH release. GABA, the dominant inhibitory neurotransmitter in the hypothalamus affects GnRH release by two different classes of membrane receptor molecules: $GABA_A$ (Rabow et al., 1995; Sieghart, 1995) and $GABA_B$ receptors (Mott and Lewia, 1994; Kaupman et al., 1997). GABA-ergic synapses have been identified on GnRH neurons (Leranth et al., 1988; Jansen et al., 2003) and GnRH cells express both type receptors (Petersen et al., 1993; Jung et al., 1998; Pape et al., 2001; Moragues et al., 2003; Sliwowska et al., 2006). It has been documented that GABA acting through $GABA_A$ as well as $GABA_B$ receptor mechanism may stimulate (Jarry et al., 1991; Feleder et al., 1996; Jackson et al., 2000; Jackson and Kuehl, 2002, 2004; Tomaszewska-Zaremba and Przekop, 2005; Moenter and De Fazio, 2005) or inhibit (Hartman et al., 1990; Akema and Kimura, 1990; Scott and Clarke, 1993a,b; Mitsushima et al., 1994; Tomaszewska-Zaremba et al., 2003a,b) GnRH release and GnRH cell activities depending upon the physiological state of animals, site of its action and experimental condition.

The GABA-ergic neurons may also serve as a primary integrating center for many different inputs to GnRH. Signals from steroid hormones (Dobson et al., 2003; Sullivan and Moenter, 2004) and neural systems (Dobson et al., 2003; Sullivan and Moenter, 2005) have been shown to alter GABA-ergic drive to GnRH neurons, which may contribute to alter in GnRH output. It is generally accepted that muscimol, a selective agonist of $GABA_A$ receptors, decreases LH secretion in sheep (Scott and Clarke, 1993a,b; Tomaszewska-Zaremba et al., 2003a,b); the results of using $GABA_A$ receptor blocker, bicuculline, on this hormone release have been variable. In the ovariectomized ewes, injection of bicuculline into the preoptic area inhibited LH secretion under all conditions except during breeding season, when LH was greatly suppressed by estrogen (Scott and Clarke, 1993a,b). Perfusion of bicuculline into the preoptic area or ventromedial hypothalamus of follicular-phase ewes had no evident effect on extracellular concentrations of GnRH in perfusates (Tomaszewska-Zaremba et al., 2003a,b). Dialysis of this drug into the preoptic area of castrated rams reduced LH release (Ferreira et al., 1998). Thus in sheep the effect of bicuculline on GnRH/LH release in high degree is dependent upon the physiological states.

It has been documented *in vitro* that activation of $GABA_A$ receptors depolarizes as well as hyperpolarizes GnRH cells (Han et al., 2002; De Fazio et al., 2002) and that the endogenous GABA can excite and inhibit the firing of GnRH neurons (Han et al., 2004; Moenter and De Fazio, 2005). Studies exploring the effect of $GABA_A$ receptor antagonist on GnRH neurons showed that majority of these cells were depolarized and/or excited by blocking of $GABA_A$ receptors (Han et al., 2004).

The mechanism underlying the effect(s) of GABA on GnRH release and GnRH cells activity is still poorly understood. Perhaps the most reasonable explanation for contradictory data may be the assumption that $GABA_A$ and $GABA_B$ receptors are located not only on GnRH cells, but also on numerous stimulatory and inhibitory interneurons that impinge on GnRH cells; the final effects of their action on GnRH cells is determined by the net results of inhibition and disinhibition