Brain Research 1650 (2016) 21–30

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Progesterone-induced amplification and advancement of GnRH/LH surges are associated with changes in kisspeptin system in preoptic area of estradiol-primed female rats

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ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form
15 August 2016
Accepted 16 August 2016
Available online 23 August 2016

Keywords:
GnRH
Gonadotropin
Estradiol
Progesterone
Hypothalamus
Kisspeptin

ABSTRACT

The time course effects of ovarian steroids on kisspeptin and GnRH/LH systems is not totally clarified. We investigated the temporal relationship among kisspeptin and GnRH mRNA and kisspeptin content in the preoptic area (POA), GnRH content and release in the medial basal hypothalamus (MBH) and plasma LH levels under different steroid treatments. Ovariectomized rats treated with oil (OVO_O), oil plus single dose of estradiol (OVO_E), oil plus single dose of progesterone (OVO_P), estradiol for 3 days plus oil (OVE_O) or estradiol for 3 days plus progesterone (OVE_P) were hourly decapitated from 10:00 to 17:00 or had the MBH microdialyzed from 09:00 to 19:00. Estradiol and progesterone acutely increased POA kisspeptin content without altering POA kisspeptin mRNA levels. Short-term exposure to both hormones stimulated MBH GnRH content, although no GnRH/LH surges had occurred. Chronic estradiol-treatment increased both kisspeptin mRNA levels and content in the POA, demonstrating that long exposure to estradiol is required to activate the whole kisspeptin synthesis machinery. This was followed by the peak in the GnRH/LH release. In estradiol-primed rats, progesterone further increased POA kisspeptin content, amplified and advanced GnRH/LH surges, with no additional change on POA kisspeptin mRNA. The data show an estradiol-induced temporal association between kisspeptin increase in the POA and GnRH/LH surges. Interestingly, the classic action of progesterone in amplifying and accelerating the GnRH/LH surges seems to occur by a mechanism which involves POA kisspeptin system.

1. Introduction

The hypothalamic gonadotropin-releasing hormone (GnRH) plays a pivotal role in the control of synthesis and release of the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). Both are the pituitary hormones that regulate ovarian function (Levine and Ramirez, 1982; Herbison, 2006). GnRH is synthesized by neurons dispersed in the preoptic area (POA) and adjacent sites in the rostral portion of the hypothalamus, then released into the hypothalamo-hypophyseal portal circulation.

A great deal of evidence suggests that the ovarian steroids regulate the neural function of GnRH neurons (Herbison, 2006; Levine, 1997). Ovarian steroids exert both stimulatory and inhibitory actions on the GnRH/LH system, depending on the phase of the cycle or the hormonal experimental condition (Herbison, 2006; Levine, 1997; Levine et al., 2001).

In the last decade, a wide range of evidence has included a new peptide, named kisspeptin, in the control of the reproductive axis. It has been shown that kisspeptin and its receptor (GPR54) play an important stimulatory role in the GnRH secretion (Han et al., 2005; Messager et al., 2005; Gottsch et al., 2004). In rodents, two principal populations of kisspeptin neurons are described; the major population of these neurons is located in the anteroventral periventricular region (AVPV) of POA (Smith et al., 2005a, 2005b), which is the main site of estradiol-positive action (Goodman, 1978; Petersen and Barraclough, 1989). Kisspeptin neurons in the AVPV express both estrogen receptor alpha and progesterone receptor (Clarkson et al., 2008) and project directly to GnRH neurons, which express GPR54 (Han et al., 2005). It has been shown that estradiol positively regulates AVPV kisspeptin expression (Smith et al., 2005b; Adachi et al., 2007; Li et al., 2007), and

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http://dx.doi.org/10.1016/j.brainres.2016.08.021
0006-8993/

progesterone seems to play a pivotal role on kisspeptin neurons during the positive feedback induction of LH surge (Stephens et al., 2015).

Since the kisspeptin/GnRH system is crucial to the reproductive processes, understanding the estradiol and progesterone actions on this system during the positive feedback mechanism becomes essential. As there is a lack of good temporal resolution concerning ovarian steroid actions on the kisspeptin/GnRH/LH system, this study aimed to report the temporal effects of different estradiol and progesterone treatments on 1) kisspeptin and GnRH mRNA and kisspeptin content in the POA, 2) GnRH content and release in the MBH and 3) the temporal association of the events involving kisspeptin and GnRH with LH surge.

2. Results

2.1. Effects of ovarian steroids on plasma LH levels and GnRH release in the MBH of ovariectomized rats

GnRH release in the MBH as well as the plasma LH levels of OVO_O, OVO_P, OVO_E, OVE_O and OVE_P rats are shown in Fig. 1. In OVO_O, OVO_P and OVO_E rats, GnRH release in the MBH (Fig. 1F, G, H) as well as plasma LH levels (Fig. 1A, B, C) did not vary over the hours sampled. In the OVE_O group, GnRH release in the MBH increased at 15:00 and 16:00 (Fig. 1I), and the plasma LH levels enhanced at 16:00 and 17:00 ($P < 0.05$; Fig. 1D), compared to the first time evaluated. In the OVE_P group, GnRH release in the MBH sharply increased at 13:00 ($P < 0.05$; Fig. 1J), and plasma LH levels were enhanced at 15:00 and 16:00 ($P < 0.05$; Fig. 1E).

2.2. Effects of single exposure to estradiol or progesterone and long exposure to estradiol in ovariectomized rats on kisspeptin and GnRH mRNA expression in the POA, kisspeptin content in the POA, GnRH content and release in the MBH, and plasma LH levels

The effects of single exposure to estradiol (OVO_E) or progesterone (OVO_P) and long exposure to estradiol (OVE_O) in ovariectomized rats on the relative expression of kisspeptin and GnRH mRNA in the POA, kisspeptin content in the POA, GnRH content in the MBH, the area under the curve (AUC) of GnRH release in the MBH and plasma LH levels over the hours sampled are shown Fig. 2.

Compared to 10:00, no changes were observed in the kisspeptin mRNA expression in the POA during the 11:00 to 16:00 period in all experimental groups (Fig. 2A). The effects of single exposure to estradiol (OVO_E) or progesterone (OVO_P) and long exposure to estradiol (OVE_O) on the relative kisspeptin mRNA expression in the POA at each time point shows that, compared to OVO_O, long exposure to estradiol increased the kisspeptin mRNA expression during the 10:00 to 16:00 period ($P < 0.001$) (Fig. 2A). Neither the acute estradiol (OVO_E group) nor progesterone (OVO_P group) treatment modified the kisspeptin mRNA expression at all times studied (Fig. 2A).

Fig. 2B shows that in OVO_O, OVO_E and OVO_P rats, compared to 11:00, the content of kisspeptin in the POA did not vary over the hours sampled. In OVE_O rats, POA kisspeptin content increased at 13:00 and 17:00 ($P < 0.05$), compared to 11:00. Comparing OVO_E, OVO_P and OVE_O groups to OVO_O at each time point, it was observed that acute treatment with estradiol increased kisspeptin content in the POA at all the time points evaluated ($P < 0.01$; Fig. 2B), while acute treatment with progesterone increased POA kisspeptin content at 11:00, 13:00 and 17:00 ($P < 0.01$; Fig. 2B). Estradiol injected for 3 days increased this neuropeptide content in the POA at 13:00, 15:00 and 17:00 ($P < 0.05$; Fig. 2B).

In OVO_O rats, POA GnRH mRNA expression was lower during the 11:00 to 16:00 period, compared to 10:00 ($P < 0.05$; Fig. 2C). Acute estradiol treatment did not modify the pattern of GnRH mRNA expression exhibited by OVO_O rats ($P < 0.05$), except at 12:00, when estradiol reversed the decrease in the GnRH mRNA expression in the POA (Fig. 2C). Likewise, acute progesterone treatment reversed the decrease in the GnRH mRNA expression in the POA at 13:00, 14:00 and 16:00 (Fig. 2C). Compared to 10:00, an increase in the POA GnRH mRNA expression induced by 3-days injection of estradiol was observed at 12:00 ($P < 0.05$; Fig. 2C).

The comparison of OVO_E, OVO_P and OVE_O groups to OVO_O on the relative GnRH mRNA expression in the POA at each time point shows that, 3-days injection of estradiol (OVE_O group) reduced the GnRH mRNA expression at 10:00 ($P < 0.01$; Fig. 2 C). Compared to OVO_O, when estradiol was acutely administrated (OVO_E group) or applied for 3 days (OVE_O group), it increased the POA GnRH mRNA expression at 12:00 ($P < 0.05$; Fig. 2C), while single progesterone injection (OVO_P) did not modify the responses shown by OVO_O animals at any time evaluated.

Fig. 2 also demonstrate that in OVO_O and OVO_P rats, GnRH content in the MBH (Fig. 2D) did not vary throughout the hours sampled. In the OVO_E group, GnRH content in the MBH increased at 15:00 and 17:00 ($P < 0.05$), compared to 11:00 (Fig. 2D). In the OVE_O group, MBH GnRH content increased at 17:00 ($P < 0.05$; Fig. 2D), compared to 11:00. Concerning the comparison of OVO_E, OVO_P and OVE_O groups to OVO_O at each time point, it could be observed an increase of GnRH content in the MBH in OVO_P at 13:00 ($P < 0.01$; Fig. 2D), and in OVO_E and OVE_O at 17:00 ($P < 0.05$; Fig. 2D), compared to OVO_O. Data of GnRH release in the MBH and plasma LH levels of OVO_O, OVO_E, OVO_P and OVE_O groups, shown in Fig. 2, were integrated and expressed as AUC, which shows that only 3-days injection of estradiol (OVE_O) reduced ($P < 0.05$) GnRH release in the MBH, compared to OVO_O group (Fig. 2E), while both acute injection of estradiol (OVO_E) and estradiol injected for 3 days (OVE_O) reduced ($P < 0.05$) plasma LH levels, compared to OVO_O animals (Fig. 2 F).

2.3. Effects of progesterone on estradiol-primed rats on kisspeptin and GnRH mRNA expression in the POA, kisspeptin content in the POA, GnRH content in the POA and MBH, GnRH release in the MBH and plasma LH levels

Fig. 3 shows the effects of estradiol-priming (OVE_O) and progesterone in estradiol-primed rats (OVE_P) on the relative expression of kisspeptin and GnRH mRNA in the POA, kisspeptin content in the POA, GnRH content in the MBH, the AUC of GnRH release in the MBH and plasma LH levels over the hours sampled. As the results concerning OVE_O group, also shown in Fig. 2, have been described in the section above, this section will describe only the time course effects in the OVE_P group, and the comparison of OVE_P with OVE_O in each time point.

Similarly to OVE_O group, compared to 10:00, no changes were observed in the kisspeptin mRNA expression in the POA during the 11:00 to 16:00 period with the administration of progesterone in estradiol-primed rats (OVE_P group; Fig. 3A). There was no difference on POA kisspeptin mRNA expression between OVE_O and OVE_P groups at each time point evaluated (Fig. 3A). Compared to 11:00, POA kisspeptin content increased at 13:00 and 15:00 ($P < 0.05$) in OVE_P animals (Fig. 3B). The comparison between OVE_O and OVE_P groups revealed that administration of progesterone to estradiol-primed rats further increased ($P < 0.05$) kisspeptin content in the POA at 11:00, 13:00 and 15:00 (Fig. 3B).

Administration of progesterone in estradiol-primed rats (OVE_P group) enhanced the GnRH mRNA expression in the POA at 15:00 ($P < 0.05$), compared to 10:00 (Fig. 3C). In addition, in the OVE_P group, progesterone blocked the increase of POA GnRH mRNA expression induced by estradiol at 12:00 (Fig. 3C). In the OVE_P group, the GnRH content in the MBH increased at 13:00 and 15:00,