REPRODUCTION

REVIEW

Unraveling the connection between GABA and kisspeptin in the control of reproduction

Noelia P Di Giorgio[1], Marianne Bizzozzero-Hiriart[1], Carlos Libertun[1,2] and Victoria Lux-Lantos[1]

[1]*Instituto de Biología y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina and* [2]*Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina*

Correspondence should be addressed to V Lux-Lantos; Email: vlux@lantos.com.ar

Abstract

Neuroendocrine control of reproduction involves the interplay of various factors that become active at some point along development. GnRH is the main neurohormone controlling reproduction and among the most important inputs modulating GnRH synthesis/secretion are GABA and kisspeptins. These interactions of GABA and kisspeptin in the control of GnRH secretion can take place by the presence of the receptors of both factors on the GnRH neuron or alternatively by the actions of GABA on kisspeptin neurons and/or the actions of kisspeptin on GABA neurons. Kisspeptin acts on the Kiss1R, a seven transmembrane domain, $G_{\alpha q/11}$-coupled receptor that activates phospholipase C, although some $G_{\alpha q/11}$-independent pathways in mediating part of the effects of Kiss1R activation have also been proposed. GABA acts through two kinds of receptors, ionotropic GABAA/C receptors involving a chloride channel and associated with fast inhibitory/stimulatory conductance and metabotropic GABAB receptors (GABABR) that are $G_{i/0}$ protein linked inducing late slow hyperpolarization. In this review, we aim to summarize the different ways in which these two actors, kisspeptin and GABA, interact to modulate GnRH secretion across the reproductive lifespan.

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Introduction

Reproduction is a fundamental feature of all known life and is essential for the perpetuation of the species. In mammals reproduction is under tight neuroendocrine control, and it involves the interplay of endocrine factors, neurons and various neurotransmitters and neuropeptides that become active at some time along development. The interaction of all these factors translates the information of environmental and internal cues into a specific timing and pattern of hormone secretion that will enable reproduction when it has the highest chances of being successful. The gonadotropin-releasing hormone (GnRH) neurons represent the final output pathway of the neuronal network controlling reproduction in all mammalian species. The regulation of GnRH secretion depends on numerous inputs into GnRH neurons, which have been the aim of intense investigations for many years. Among these many inputs, the salient ones include kisspeptin (de Roux *et al.* 2003, Seminara *et al.* 2003), GABA (Donoso & Banzan 1986, Akema & Kimura 1991, Robinson *et al.* 1991, Keen *et al.* 1999) and glutamate (Gay & Plant 1987, Urbanski & Ojeda 1987, Suzuki *et al.* 1995, Gore *et al.* 1996).

GABA is the main inhibitory neurotransmitter in the mammalian central nervous system. It acts through two kinds of receptors, ionotropic GABAA/C receptors involving a chloride channel and associated with fast inhibitory/stimulatory conductance events, and metabotropic GABAB receptors (GABABR) that are $G_{i/0}$ linked inducing late, slow hyperpolarization, diminution in membrane Ca2+ conductance by voltage-sensitive calcium channels (VSCC), increase in membrane K+ conductance (Kir-3) and inhibition of adenylyl cyclase. Pharmacologically, these receptors differ in their response to agonists and antagonists.

GABAARs are agonized by muscimol and antagonized by bicuculline, gabazine, picrotoxin, PHP 501 trifluoroacetate, SR95531, among others.

GABAB receptors are typically agonized by baclofen and antagonized by phaclofen, 20-hydrxysaclofen, CGP55845, CGP52432, SCH50911, among others.

It is now well established that GABA plays more than an inhibitory role and can function as an important stimulatory developmental signal early in life (Lauder *et al.* 1998, Ziskind-Conhaim 1998, Fiszman & Schousboe 2004, Galanopoulou 2008, Tobet *et al.* 2009, Kilb *et al.* 2013). In the study of GABA as a neurotransmitter, neurohormone or neurotrophic signal, its actions through the GABAAR have gained maximal attention, while its actions through the GABABR have been less well studied. Nevertheless, GABA acting on GABABRs has many important effects that are slowly coming to light. For example, it has been recently demonstrated that activation of metabotropic GABABRs in cortical layer 1 can powerfully inhibit principal

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cell activity and that their activation can rapidly halt ongoing network activity (Craig & McBain 2014). In addition, GABABRs promote radial migration into the upper cortical plate and tangential migration of interneurons (Luhmann *et al.* 2015). GABABRs also regulate crucial steps of neuronal network formation, including migration, neurite growth, synapse formation and plasticity (Gaiarsa & Porcher 2013).

GABA contributes to the neural circuits that control reproduction during the whole life span. GABAergic afferents are known to be among the major synaptic inputs to GnRH neurons (Leranth *et al.* 1985). There are conflicting reports on whether GABA is stimulatory or inhibitory on GnRH release. While the net effect of increased GABA within the POA seems to inhibit GnRH secretion (Han *et al.* 2004), direct action of GABA on GnRH neurons induced GABAAR-mediated excitation (DeFazio *et al.* 2002). Currently, there is growing consensus that GABA can act through the GABAARs to exert both depolarizing and hyperpolarizing effects on GnRH neurons, but the predominant action seems to be that of excitation, while the effect of GABAB receptor stimulation is always inhibitory (Herbison & Moenter 2011).

GABA participates in the migration of GnRH neurons from the olfactory placode into the hypothalamus during embryonic development (Fueshko *et al.* 1998, Tobet *et al.* 2001, Wierman *et al.* 2011, Gaiarsa & Porcher 2013). Moreover, it contributes to puberty onset and the regulation of the preovulatory LH surge in adult females (Jarry *et al.* 1995, Terasawa & Fernandez 2001, Clarkson & Herbison 2006, Maffucci & Gore 2009, Moenter *et al.* 2009). Some of these effects are exerted directly on GnRH neurons, while others are exerted by interneurons as, for example, kisspeptin neurons.

Kisspeptin, encoded by the *Kiss1* gene, plays an essential role in the control of reproduction as has been shown by the groundbreaking works of de Roux *et al*. and Seminara *et al*. who demonstrated in 2003 that inactivating mutations of the GPR54 (*Kiss1r*) gene were associated with hypogonadotropic hypogonadism in humans and mice (de Roux *et al.* 2003, Seminara *et al.* 2003). Since then, kisspeptin signaling has been identified as one of the critical regulators for both puberty onset and maintenance of normal reproduction in mammals, as well as also participating in metabolic control and behavior, among other functions (Kaiser & Kuohung 2005, Dungan *et al.* 2006, Seminara 2006, Roa *et al.* 2008, Kauffman & Smith 2013, Beltramo *et al.* 2014, Millar & Babwah 2015, Ronnekleiv *et al.* 2015, Liu & Herbison 2016, Lehman *et al.* 2018).

In adult rodents kisspeptin is mainly expressed in two hypothalamic regions, the anteroventral periventricular nucleus and neighboring periventricular nucleus (AVPV/PeN) and the arcuate nucleus (ARC). *Kiss1* expression in the AVPV/PeN is sexually dimorphic, being more abundant in adult females than in males, while *Kiss1* expression in the adult ARC does not show evident sex differences (Semaan *et al.* 2013). Additional extrahypothalamic regions of *Kiss1* expression have been identified such as the lateral *septum* (LS), bed nucleus of the *stria terminalis* (BNST) and the medial amygdala (MeA) (Clarkson *et al.* 2009, Cravo *et al.* 2011, Kim *et al.* 2011, Di Giorgio *et al.* 2014, Pineda *et al.* 2017), areas with an important role in the modulation of various social and sexual behaviors.

As we mentioned earlier, both kisspeptin and GABA are important modulators of the hypothalamic–hypophyseal–gonadal axis. Here, we aim to unravel the interplay between GABA and kisspeptin in the control of reproduction by addressing the effects of GABA on both GABAA and GABAB receptors, mainly from evidence in rodents. These interactions in hypothalamic nuclei will be examined in three developmental phases: perinatal, peripubertal and adult. Kisspeptin and GABA interactions will also be analyzed in extra-hypothalamic sites that modulate reproduction in several ways such as sexual behavior.

Interactions of kisspeptin and GABA in the hypothalamus

Perinatal period

It has been postulated that perinatally *Kiss1* expression is detected in the ARC but not so readily in the AVPV/PeN, where it only appears at approximately postnatal day 10 (PND10) and starts to show the sexually dimorphic expression (females > males) on PND12 (Semaan *et al.* 2013). Nevertheless, more recent work demonstrated the presence of a male-specific population of pre-optic area kisspeptin neurons that appear transiently in the perinatal period: neurons positive for kisspeptin protein were first detected on E17, increasing in number over E19 to reach a peak on the day of birth before declining to zero on PND5. These kisspeptin neurons provide one possible source of kisspeptin drive to neonatal GnRH neurons and consequent neonatal testosterone production in the mouse that is fundamental for the sexual differentiation of the brain after aromatization to estradiol (Clarkson & Herbison 2016). The fast decline in these neurons is probably a consequence of the perinatal testosterone surge they elicit.

Regarding the ARC, expression of *Kiss1* mRNA in embryonic rodents was determined in both sexes around E11.5 in rats and E13 in mice and increased by E18.5, with a sharp drop in levels just prior to birth (Semaan *et al.* 2013). Although there are no consistent sex differences in ARC *Kiss1* expression in adulthood, early postnatally ARC *Kiss1* mRNA expression is higher in females than in males and this lasts from birth until the juvenile period begins (PND11). Whether these early sex differences are also observed during embryological life remains controversial. Perinatal male testosterone

secretion also induces the early loss of ARC *Kiss1* expression in males (Semaan *et al.* 2013), allowing for the sex differences observed early postnatally.

Interestingly, one of the mediators of the sex-differentiating actions of testosterone/estradiol is GABA (McCarthy *et al.* 2002), particularly in the ARC (McCarthy *et al.* 2008).

In addition to its sex-differentiating effects, and as we mentioned earlier, GABA modulates GnRH neurons early during embryogenesis by modulating their migration from the olfactory placode into the hypothalamus (Fueshko *et al.* 1998, Tobet *et al.* 2001, Wierman *et al.* 2011, Gaiarsa & Porcher 2013). These GnRH neurons express both GABAA and GABAB receptors (Tobet *et al.* 2001). Whether a similar interaction between GABAergic and *Kiss1* neurons exists at this early stage of development remains unknown. Adult *Kiss1* neurons express GABAA (Ducret *et al.* 2010, Gottsch *et al.* 2011, Alreja 2013) and GABAB receptors (Di Giorgio *et al.* 2014). However, it is not known when *Kiss1* neurons commence to express these receptors and to be modulated by GABA.

A recent study evaluated the effect of GABAAR activation on *Kiss1* gene expression in a rat hypothalamic kisspeptin-producing cell line derived from immortalized E18 hypothalamic primary cultures, the rHypoE8 cells and in primary cultures of the neuronal cells of fetal rat brains. They found that muscimol could actually stimulate *Kiss1* gene expression in both models (Kanasaki *et al.* 2017). Interestingly, when rHypoE8 cells were stimulated with GABA instead of muscimol, this stimulatory effect was not observed, suggesting that GABABRs also activated by GABA may have a contrary effect on *Kiss1* expression. These results hint to active GABA receptors at this developmental age; nevertheless, in these models, a direct action of GABA on kisspeptin neurons cannot be asserted, as in both cases, the cell populations did not consist exclusively of kisspeptin neurons.

We have observed that at PND4 the well-described sex difference in ARC *Kiss1* expression (females > males) was abolished in mice lacking functional GABAB receptors (GABAB1KO). *Kiss1* mRNA expression was downregulated in females attaining male levels (Di Giorgio *et al.* 2013). Interestingly, at this postnatal age, we found increased AVPV/PeN *Kiss1* mRNA expression in males with regard to females, in agreement with Clarkson and Herbison (Clarkson & Herbison 2016), without any genotype difference. These results suggest a possible participation of GABABRs in establishing nucleus-specific sex difference in *Kiss1* expression, with effects in the ARC but not in the AVPV/PeN. In order to evaluate GABAB receptor effects in ARC kisspeptin expression by a pharmacological approach, BALB/c pups were injected from PND2 to PND6 with the GABAB antagonist CGP55845 or with saline and were killed on PND6. *Kiss1* mRNA expression in ARC punches showed the expected sex difference in saline-treated mice (females > males). CGP55845 induced significant downregulation of *Kiss1* expression in both males and females (Bizzozzero *et al.* 2017). However, in CGP55845-injected mice, the sex difference was maintained, showing somewhat different effects from those observed in GABAB1KO mice, in which GABABRs are missing from conception onward. No effect on AVPV-PeN *Kiss1* expression was observed by treatment with the GABAB antagonist, reinforcing that the modulation by GABABRs is nucleus specific. Again, in both these genetic and pharmacological approaches, direct actions of GABA on kisspeptin neurons cannot be verified.

Interestingly, *Kiss1* expression in the APVP/PeN and ARC of adult GABAB1KO mice is similar to WTs (Di Giorgio *et al.* 2014). Moreover, CGP55845 injection to adult mice did not modify *Kiss1* expression in either nucleus (Bizzozzero *et al.* 2017), demonstration that the effect of GABAB receptor modulation on ARC *Kiss1* expression depends on the stage of development. As adult GABAB1KO mice have compromised reproduction (Catalano *et al.* 2005), we postulate that the distortion in neonatal ARC *Kiss1* expression may influence the programming of the reproductive network. This hypothesis is currently under investigation.

To our knowledge, there is no description of direct GABA actions on kisspeptin neurons modifying kisspeptin expression, synthesis, secretion or function at this early developmental phase.

Peripubertal period

In rodents, AVPV/PeN *Kiss1* expression shows a steady and continuous increase in cell number from PND15 through PND28, at which point it attains adult levels; the sex difference (females > males) was first detected at PND12 and robustly observed as from PND16 (Semaan *et al.* 2013).

In the ARC, the number of *Kiss1* neurons rise moderately over the pubertal transition reaching adult levels around the time of vaginal opening in females, with older pubertal ages (PND28–30) being significantly higher than earlier pubertal ages (PND20–22) (Semaan & Kauffman 2015). As mentioned earlier, in the adult ARC, *Kiss1* mRNA expression is not majorly different between males and females, especially when circulating sex steroid levels are equalized between the sexes (Semaan *et al.* 2013). Nevertheless, some studies show increased ARC kisspeptin immunoreactivity in adult gonadally intact and gonadectomized female mice with regard to males (Budefeld *et al.* 2016).

Regarding the interaction of GABA and kisspeptin during this stage of development, in monkeys, it has been proposed that there is a central restrain of GnRH release prior to puberty and that GABA, acting on GABAAR, is a crucial component of this inhibition. Blocking GABAAR in prepubertal monkeys stimulated kisspeptin release in the medial basal hypothalamus that in turn

stimulated GnRH secretion, but this was not observed after puberty onset, suggesting that there is a decline in GABA inhibition at this time point. These results suggest that kisspeptin neurons may relay inhibitory GABA signals to GnRH neuron before puberty (Terasawa *et al.* 2010, Kurian *et al.* 2012). A similar mechanism is also proposed to be active in humans (Check 2013). Whether a similar mechanism is also present in rodents is still under investigation.

Regarding GABAB receptors, female GABAB1KO mice showed normal puberty onset (Catalano *et al.* 2005), suggesting that GABAB receptors do not participate in this process.

Adulthood

Most of the work on the interaction of GABA and kisspeptin in the control of reproduction has been performed in adult mice, especially in females. An early electrophysiological work indicated that kisspeptin activated GnRH neurons via both direct and transsynaptic mechanisms (Pielecka-Fortuna *et al.* 2008). Transsynaptic mechanisms were either enabled and/or potentiated by estradiol. The AVPV/PeN expresses *Kiss1r*, and this region is rich in GABAergic, glutamatergic and dual GABA/glutamatergic neurons that project to GnRH neurons. Pielecka-Fortuna *et al*. demonstrated that kisspeptin increased GABAergic and glutamatergic transmission directly to GnRH neurons in an estradiol-dependent manner and that kisspeptin increased GnRH neuron response to activation of GABAA receptors (Pielecka-Fortuna & Moenter 2010), demonstrating that kisspeptin modifies GABAergic transmission to GnRH neurons at the time of the preovulatory surge.

Interaction of kisspeptin with GABA was further proposed by Neal-Perry *et al*. who showed that specifically, age-related changes in LH surge amplitude may be causally linked to reduced *Kiss1* mRNA expression in the AVPV, reduced mPOA glutamate and increased mPOA GABA release (Neal-Perry *et al.* 2009). Interestingly, kisspeptin infusion into the mPOA of estradiol-primed rats rescued GnRH/LH release and elevated local glutamate and decreased local GABA release, thereby restoring the balance of local excitatory and inhibitory amino acid release in the mPOA of middle-aged rats to levels typical of young females. Thus, an inhibitory effect of kisspeptin on GABA neurons is suggested here, at least in this particular animal model of middle-aged rats. Together, these results suggest that the effect of kisspeptin on GABA release may depend on the endocrine milieu.

It has been suggested that *Kiss1* neurons may also coexpress additional neurotransmitters. Using transgenic mice Cravo *et al.* (2011) described that in AVPV/PeN approximately 20% of *Kiss1* neurons coexpressed *vGluT2* mRNA, a marker for glutamatergic neurons, and 75% coexpressed *Gad1* mRNA, a marker for GABAergic neurons. In the AVPV/PeN neurons expressing the three genes, *Kiss1*, *Gad1* and *vGlutT2* mRNAs, were also observed. Therefore, neurons containing GABA and kisspeptin may simultaneously or sequentially secrete these neurotransmitters to modulate GnRH neurons. In the ARC, 90% of *Kiss1* neurons coexpressed *vGluT2* mRNA and 50% of these also coexpressed *Gad 1*mRNA. However, another more recent study, described very limited coexpression of kisspeptin and GABA in ARC neurons in both males and females (Marshall *et al.* 2017).

Further electrophysiological studies on the impact of AVPV/PeN stimulation on GnRH neuron excitability (Liu *et al.* 2011) demonstrated that a high percentage of GnRH neurons located in the rostral POA received monosynaptic inputs from this area. AVPV/PeN stimulation at low frequencies (1 Hz) generated short-latency action potentials in GnRH neurons with GABA and glutamate mediating 90% of the evoked fast synaptic currents. They found that the AVPV/PeN GABA input through GABAARs was dominant and excited or inhibited GnRH neurons in a cell-dependent manner. Increasing the AVPV/PeN stimulation frequency to 5–10 Hz resulted in the appearance of additional post stimulus inhibitory response, attributed to GABABRs, as well as a delayed excitatory response that was mediated by kisspeptin. In this study, it could not be established whether GABA, glutamate and kisspeptin release originated from the same neuron or from independent sets of neurons. In a very recent follow-up on these studies, Piet *et al.* demonstrated with a state-of-the-art optogenetic approach that there is effectively an AVPV/PeN neuron population that coexpresses kisspeptin and GABA and that this population provides an important double excitatory input to GnRH neurons with kisspeptin being the dominant stimulus in activating GnRH neurons at the time of ovulation (Piet *et al.* 2018).

In addition to kisspeptin modulating GABA neurons and the existence of neurons expressing both kisspeptin and GABA, there exists also evidence that GABA can modulate kisspeptin neurons.

When analyzing the actions of GABA on AVPV/PeN and ARC *Kiss1* neurons, an inhibition of *Kiss1* neuron firing was observed when GABAARs were activated (Ducret *et al.* 2010, Gottsch *et al.* 2011, Alreja 2013); this could be relevant to various reproductive states including the estrous cycle. In hypothalamic slices from animals with GFP-marked kisspeptin neurons, DeFazio *et al.* (2014) demonstrated that both estradiol and time of day influenced GABAergic transmission to hypothalamic kisspeptin neurons as well as their response to GABA through GABAARs. While GABA generally hyperpolarized AVPV/PeN kisspeptin neurons, there was an estradiol-induced decrease in GABAergic input to these neurons in the afternoon of ovariectomized estradiol-treated (OVX-E) mice, allowing for the increase in kisspeptin output. Conversely, GABAAR stimulation induced

depolarization of ARC kisspeptin neurons although not likely to induce excitation. In the presence of estradiol this depolarization could be sufficient to inactivate voltage-gated sodium channels and result in an increased threshold for action potential firing ('depolarizing inhibition'), consistent with the current view that steroid negative feedback is conveyed to GnRH neurons at least in part by inhibition of ARC kisspeptin neurons. These estradiol-modulated effects of GABA on both kisspeptin neuron populations may be critical for eliciting the preovulatory LH surge.

Additional work suggested a critical role for GABA in the elicitation of the preovulatory kisspeptin/GnRH/LH surges. Leon *et al.* working with global Grp54 KO mice and with GnRH Gpr54−/−Tg-rescued mice, in which the *Gpr54* gene was selectively reintroduced into GnRH neurons, provided conclusive evidence that the GABAA receptor blockade-induced increase in LH secretion is completely dependent on kisspeptin signaling to GnRH neurons, as LH secretion was abolished in global Grp54 KO and recovered in GnRH Gpr54−/−Tg rescued mice (Leon *et al.* 2016).

A very interesting work evaluated the effect of GABA, acting on GABABRs, on kisspeptidergic neurons. It has been shown that burst firing facilitates neuropeptide release from neurons. In order to attain a 'preovulatory' release of kisspeptin these neurons probably depend on burst firing. While studying the biophysical properties of *Kiss1* neurons in the AVPV-PeN, Zhang *et al.* (2013) observed that the crucial T-type calcium current necessary for rebound burst firing was present in these *Kiss1* neurons and that this phenomenon was estrogen dependent. The presence of a robust T-current is essential for the high-frequency rebound bursting that is manifested following a hyperpolarizing stimulus, and they demonstrated that this hyperpolarizing stimulus was delivered to *Kiss1* neurons by GABABR agonists, such as baclofen, and also by μ- and κ-opioid agonists, suggesting that these pathways contribute to the preovulatory release of kisspeptin (Zhang *et al.* 2013). They confirmed the presence of μ and κ opioid receptor mRNA in these *Kiss1* cells. Consistent with the effect they described with a GABAB agonist, 98% of AVPV-PeN *Kiss1* neurons express the *Gabab1* subunit mRNA of the GABAB receptor (Di Giorgio *et al.* 2014). Adult GABAB1KO females showed normal *Kiss1* expression in the AVPV-PeN and ARC; however, they have compromised reproduction (Catalano *et al.* 2005). Although *Kiss1* expression was not altered in the AVPV-PeN, taking into consideration the results observed by Zhang *et al.* (2013) described earlier, the absence of GABAB signaling to *Kiss1* neurons may jeopardize the hyperpolarizing input to kisspeptidergic neurons necessary for the high-frequency rebound bursting activity contributing to the preovulatory kisspeptin surge. This hypothesis is currently under investigation in our laboratory.

An alternative way in which GABABRs and kisspeptin interact in the control of reproduction is directly at the GnRH neuron that expresses both GABABRs and KISS1R. On the one hand, the GABABR agonist baclofen hyperpolarized GnRH neurons through activation of an inwardly rectifying K+ current (Kir) in a concentration-dependent manner, providing an inhibitory tone. On the other hand, kisspeptin depolarizes GnRH neurons through $G_{\alpha q/11}$-phospholipase C signaling-mediated inhibition of these same Kir channels as well as through activation of a canonical transient receptor potential (TRPC)-like cationic channel (Zhang *et al.* 2008). Therefore, in the presence of estradiol, such as in proestrus, strong kisspeptin stimulation will attenuate the GABAB inhibitory input, as they use the same mechanism in opposite directions to exert their effects (Zhang *et al.* 2009).

Reproduction and metabolism are closely related, and it has been demonstrated that leptin is a major participant in this coordination (Barash *et al.* 1996, Chehab *et al.* 1996, de Luca *et al.* 2005). When trying to identify the nature of leptin-responsive neurons mediating these effects in female mice, Martin *et al.* (2014) demonstrated that leptin-responsive GABAergic neurons, but not glutamatergic neurons, act as metabolic sensors to regulate fertility. Lack of leptin receptors on GABAergic neurons impaired reproduction and normal kisspeptin response to OVX in the ARC and to OVX-E in the AVPV/PeN. These findings suggested that the reduction of the inhibitory GABAergic tone on kisspeptin neurons necessary for puberty progression and for preovulatory GnRH-LH surges is in part elicited by leptin signaling on GABAergic neurons, thus linking energy homeostasis with reproduction.

In brief, all these studies clearly demonstrate that GABA interacts with kisspeptin to regulate reproduction in a variety of ways along development. This relationship is bidirectional, that is, kisspeptin modulates GABAergic transmission and GABA modulates kisspeptidergic transmission, in addition to coexpression of both factors in the same neuron, all of which finally impact GnRH neuron physiology. Whether this bidirectional interaction between kisspeptin and GABA is also reciprocal remains to be established.

Interactions of kisspeptin with GABA in extra hypothalamic sites

As mentioned earlier, kisspeptin neuron populations outside the hypothalamus have also been described in areas that can affect reproduction in different ways.

Li *et al.* (2015) demonstrated that antagonism of GABAAR specifically in the medial posterodorsal amygdala (MePD) advanced the timing of puberty, implying that normally the GABAergic input from this area restrains puberty onset; the opposite effect

was true of glutamatergic input. These results, together with previous observations, suggest that the amygdala, an area involved in controlling many social and reproductive behaviors, participates in pubertal awakening through its GABAergic and glutamatergic neural systems.. The amygdala sends efferents to the mPOA, rich in GnRH neurons, and also to the AVPV/PeN and ARC, rich in kisspeptin neurons, through which it could exert its effects. In addition, the MeA also contains kisspeptin neurons (Kim *et al.* 2011) involved in the regulation of various aspects of reproduction such as puberty timing, male sexual behavior, modulation of gonadotropin release and pulsatility (Comninos *et al.* 2016, Gresham *et al.* 2016, Adekunbi *et al.* 2017, Pineda *et al.* 2017, Comninos & Dhillo 2018) that could be modulated by intra-amygdala GABA and glutamate.

Several studies propose the existence of various types of GABA neurons in the amygdala (Veinante *et al.* 1997, Bian 2013, Keshavarzi *et al.* 2014). A very recent work from Aggarwal *et al*. demonstrated that 71% of MeA kisspeptin neurons also coexpress Vgat, a marker for GABAergic neurons; these authors also demonstrated that this population of kisspeptin neurons process sexually relevant olfactory signals to influence reproductive hormone levels in male mice (Aggarwal *et al.* 2018). Our results show that 66% of detectable *Kiss1* neurons in the MeA coexpress *Gabab1* subunit mRNA of the GABAB receptor (Di Giorgio *et al.* 2014). Interestingly, a dramatic increase in *Kiss1* expression was observed in extrahypothalamic regions, such as the MeA, of GABAB1KO mice of both sexes. These increased extrahypothalamic *Kiss1* levels in GABAB1KO mice were not sex steroids dependent and appeared after puberty. These findings suggested that GABABR signaling may normally inhibit *Kiss1* expression in the MeA of adult mice (Di Giorgio *et al.* 2014). Our findings also indicate that both E2 and GABA, through GABABRs, independently regulate extra-hypothalamic *Kiss1* cell populations (Stephens *et al.* 2018). As GABAB1KO females have impaired reproduction, a marked increase in MeA kisspeptin input may participate in the alterations observed. It is our aim to further analyze the effect of GABABRs on *Kiss1* neurons in these extra-hypothalamic areas in the new *Kiss1*-GABAB1KO mice, lacking GABABRs exclusively in *Kiss1* cells, recently developed in our laboratory (Di Giorgio NP, Tabares F, Bizzozzero M, Bourguignon NS, Bettler B, Libertun C, and Lux-Lantos V, poster presentation ICN, Toronto 2018).

All these results propose that kisspeptin and GABA also interact in the MeA to regulate various aspects of reproduction. The physiological relevance of this interaction in other extra hypothalamic kisspeptin-expressing nuclei such as the BNST or the LS is still unknown.

Conclusion

In sum, these findings demonstrate that GABA, acting on both its GABAA and GABABRs, interacts with kisspeptin in the regulation of reproduction. Salient examples of these interactions are the following:

(a) Early in development, GABA seems to modulate Kiss1 expression: in embryonic stages, GABA acting on GABAA receptors increases *Kiss1* expression, and early postnatally GABAB receptors also seem to increase *Kiss1* expression in the ARC but not in the AVPV/PeN, as in the absence of GABAB signaling the expression declines. Whether GABA exerts these effects directly on kisspeptin neurons is currently unknown.

(b) In the prepubertal period, central restrain of GnRH release is mediated by GABA acting on GABAAR on kisspeptin neurons in monkeys and humans. Whether this mechanism is also present in rodents is still under investigation.

(c) In adults, these interactions have been more extensively investigated and are more complex. Interesting examples postulate that the AVPV/PeN neuron population that coexpresses kisspeptin and GABA provides an important double excitatory input to GnRH neurons at the time of ovulation and also that the hyperpolarizing stimulus needed for preovulatory rebound burst firing of kisspeptin neurons is mediated by GABAB receptors (and also μ and K opioid receptor); therefore, absence of GABAB signaling at this critical time may compromise the preovulatory surge.

(d) Antagonism of GABAAR specifically in the medial amygdala advanced the timing of puberty probably by disinhibiting kisspeptin neurons. Moreover, a high percentage of MeA kisspeptin neurons are also GABAergic in nature and they convey sexually relevant olfactory signals to influence reproductive hormone levels. Nevertheless, amygdala interactions of kisspeptin and GABA in the control of reproduction need further investigation.

As shown, these interactions differ depending on the kisspeptin neuron population in question and the stage of development. Alterations in each of these populations can lead to various physiopathological consequences in the reproductive axis.

In addition, especially regarding GABABRs, much information is still missing on: (a) how they regulate *Kiss1* expression, and whether this is age dependent, (b) when *Kiss1* neurons start expressing GABABRs and whether they can influence neurite growth, synapse formation and plasticity in these neurons, as demonstrated in other systems, (c) whether the absence of the hyperpolarizing effects of GABABR stimulation on *Kiss1* neurons is compensated by the action of opioids or whether it

disturbs the triggering of the preovulatory kisspeptin surge. All these questions warrant further studies that will enhance our understanding of the neural control of reproduction.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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