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Signaling of Cytokines is Important in Regulation of GnRH Neurons

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Abstract Cytokines encompass a broad class of peptides that mediate signals in a broad range of physiological situations including inflammation, infection, and obesity. The cytokine receptor-associated tyrosine kinase, Jak2, is one of the most important proteins mediating cytokine signaling pathway activation. Recently, our group has demonstrated that Jak2 signaling in the gonadotropin-releasing hormone (GnRH) neuron plays a critical role in fertility in males and females, implicating cytokine activation of the neuron in GnRH neuronal development and function. To date, the specific cytokine(s) essential for activating Jak2 during neuroendocrine development are not known. In this article, we review the evidence for the role of several class 1 cytokines in regulating GnRH neuronal development, GnRH secretion, and GnRH expression.

Keywords Cytokines · GnRH · Jak2 · Fertility · Signaling

Introduction

Cytokines are a large and diverse family of regulators secreted by the glial cells of the nervous system and by numerous cells of the immune system. An emerging concept is that there is a bidirectional interaction between the immune system and the neuroendocrine system with cytokines as the primary signals from the immune system [1]. The term "cytokines" is used to refer to immunomodulating agents such as interleukins (IL), lymphokines, monokines, chemokines, tumor necrosis factors (TNF), interferons, and growth factors (GF) [2]. Substantial evidence indicates that cytokines are produced within the mammalian central nervous system (CNS) in cells such as astrocytes [3]. These brain-derived cytokines may function to regulate specific challenges and may modulate reproduction by exerting effects directly on the hypothalamic–pituitary–gonadal (HPG) axis [3]. Gonadotropin-releasing hormone (GnRH) neurons are found scattered across the basal forebrain and secrete the decapeptide GnRH which serves to regulate reproductive function. Potential interactions between cytokines and GnRH neurons may be complicated; cytokines may act directly through their receptors on the GnRH neurons or through the release of intermediaries such as prostaglandins, nitric oxide, neuropeptides, or neurotransmitters [4]. The main focus of this review is on cytokine regulation at the level of the GnRH neuron, with special emphasis on IL, TNF, leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF) as these cytokines have been proposed to directly regulate the function of the GnRH neuron.

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GnRH Neurons Play a Pivotal Role in the Regulation of Reproductive Function

The GnRH neurons represent the final common pathway for the regulation of reproduction by the brain. GnRH neurons secrete the GnRH decapeptide which then travels via the portal vasculature to the anterior pituitary. GnRH stimulates the synthesis and secretion of the pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), from the gonadotrophs of the anterior pituitary. LH and FSH in turn stimulate the development and maturation of the gonads and direct the synthesis and secretion of the gonadal steroid hormones: testosterone from testes and

estradiol and progesterone from ovaries. GnRH neurons integrate signals that regulate reproduction such as nutrition, stress, developmental cues, and seasonal and circadian information. These stimuli exert their effects by regulating the expression and secretion of GnRH [5–9]. Environmental, homeostatic, psychogenic, and metabolic cues that could potentially signal through cytokines include inflammation, chronic disease, and obesity.

Janus kinase/signal transducer and activator of transcription (Jak/STAT) signaling plays a pivotal role in cytokine signaling [10, 11]. The Jak family consists of Jak1, Jak2, Jak3, and Tyk2 and is activated by ligands such as cytokines, growth factors, and hormonal factors binding to their cell surface receptors [10]. Jak2 is the predominant Jak kinase mediating the responses to cytokines. Once the associated cell surface receptor is activated, Jak2 is autophosphorylated on tyrosines 1007/1008 and phosphorylates the associated receptor. Phosphorylated receptors provide multiple docking sites that recruit STAT proteins. STAT is subsequently phophorylated by Jak2 resulting in dimerization and translocation to the nucleus as a transcription factor to regulate gene expression [10, 11]. There are eight genes encoding six STAT proteins: STAT1, STAT2, STAT3, STAT4, STAT5, and STAT6 [12].

Recently in our laboratory, based on the accumulated evidence for cytokine signaling in the GnRH neurons (described below), we conditionally knocked out the Jak2 gene in GnRH neurons in mice (Jak2 G−/−) and observed that female mice experienced delayed puberty and impaired fertility (Fig. 1a–c) [13]. Interestingly, there was no difference between male Jak2 G−/− and control littermates in the age of puberty as assessed by preputial separation (Fig. 2a), suggesting that there are fundamentally different mechanisms regulating puberty in males and females. We also observed sexual dimorphism in the effect of IGF1 on puberty in mice [14] further indicating that pubertal timing in males and females are regulated by different mechanisms. Male Jak2 G−/− mice did however exhibit impaired fertility with decreased numbers of pregnancies with mating (Fig. 2b, c). The reduced fertility may be due to decreased LH hormone level (Fig. 3a) as had been previously demonstrated in the female [13]. Testes weights were not significantly different between Jak2 G−/− and control littermates, and histological examination showed grossly normal development of the testes with normal morphology of spermatids, Sertoli cells, and Leydig cells (Fig. 3b, c). Jak2 G−/− mice produced the same number of pups per litter as control males (Fig. 2d), suggesting that the defect in neuroendocrine function may reduce the frequency of testosterone-dependent mating behavior, but that sperm development and delivery are not impaired in the Jak2 G−/− male mice. While LH levels are low in knockout male mice (Fig 3a), they are high enough

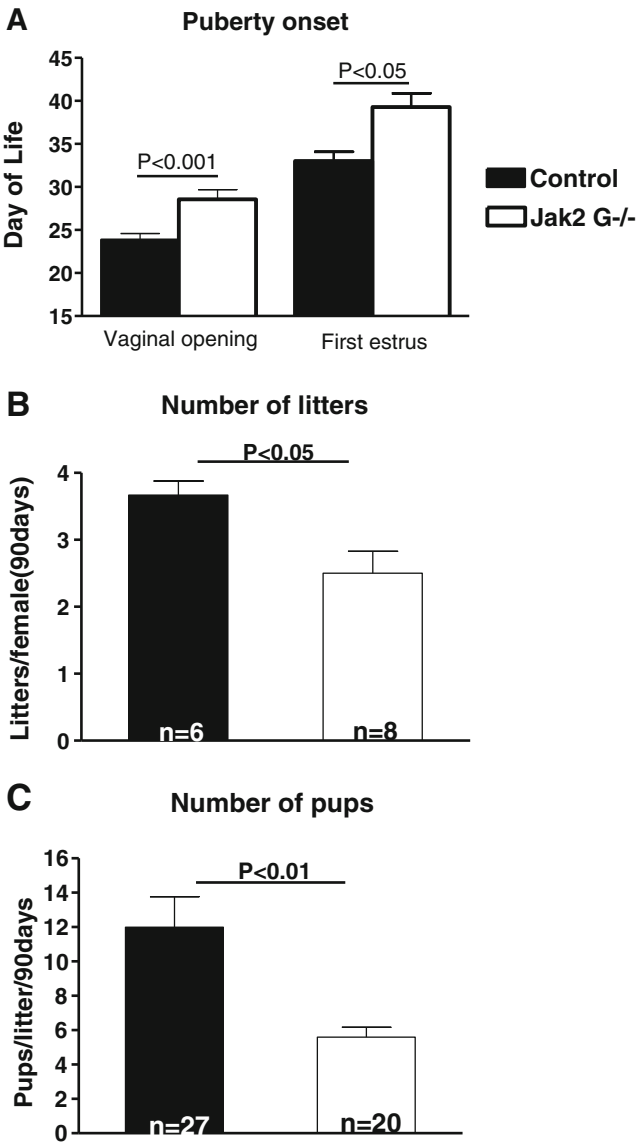

Fig. 1 Phenotype of Jak2 G−/− female mice [13]. **a** Vaginal opening was examined daily from 20 days of age in both control and Jak2 G−/− female mice and the day of opening was documented and average values displayed. Significance is indicated. **b** Female mice were mated with wild-type male mice for 90 days. Total numbers of litters per female was significantly reduced in Jak2 G−/− mice compared to control group during the 90 days. **c** Number of pups per litter was significantly reduced in Jak2 G−/− female mice compared to controls. For **a**–**c**, values are mean±SEM and significance and numbers of animals examined are indicated

to stimulate production of sufficient levels of free testosterone for sperm production. Unlike most other mammals, mice have very low levels of androgen-binding protein in the testes [15] which results in lower total testosterone requirements for spermatogenesis.

Although we demonstrated that cytokine signaling in GnRH neurons is important for reproductive function, the identity of the cytokine or combinations of cytokines that

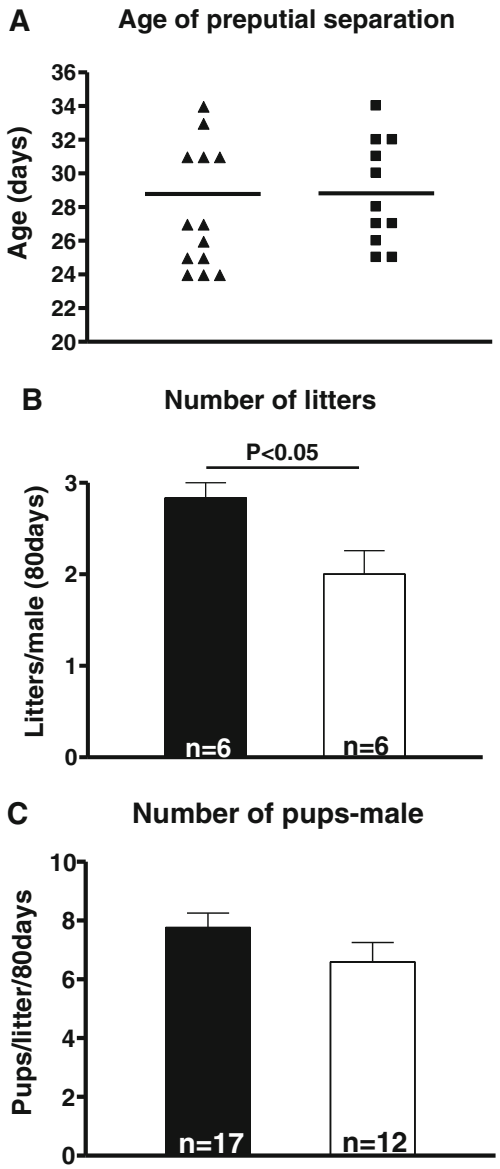

Fig. 2 Phenotype of Jak2 G−/− male mice. Male puberty was assessed daily by preputial separation after 20 days of birth as previously described [56]. Male Jak2 G−/− mice were mated with proven fertile female mice for a period of 80 days. Time to each litter and litter size for each pair were recorded. **a** Preputial separation was examined as a measure of male puberty and the day of separation was documented and average values displayed. Significance is indicated. **b** Male mice mated with wild-type female for 80 days. Number of litters per male during 80 days of mating was reduced significantly in Jak2 G−/− mice compared to controls. **c** Number of pups per litter is not significantly different between Jak2 G−/− male mice and controls. For **a**–**c**, values are mean±SEM and significance and numbers of animals examined are indicated

are activating this signaling pathway during development is not yet established. Several candidates have been proposed and the evidence for each of these cytokines in regulating GnRH neuronal development, GnRH secretion, and GnRH expression is discussed below.

Cytokines Effects on GnRH Neurons

The interleukins are a subset of a family of cytokines that modulate cellular behavior. They are produced and secreted by a host of cells that involved in immunity and other physiological functions.

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine and binds its receptors (IL-R1 and/or IL-R2) to activate mitogen-activated protein (MAP) kinase, NF-kB, and Jak2/STAT pathways [16–18].

GnRH Secretion IL-1β and its receptor are expressed in the human and rat hypothalamus [19–21] and is the most potent inhibitor of GnRH secretion that has been identified [3, 21]. It is believed to interfere with GnRH secretion at the level of GnRH perikarya rather than at the level of GnRH nerve terminals of the median eminence [3, 4, 22] and IL-1β has also been shown to reduce the spontaneous expression of c-fos protein located within the nuclei of GnRH neurons during the preovulatory gonadotropin surge in proestrus rats [4]. IL-1β has also been shown to attenuate LH levels in gonad-intact females and castrated males but not intact male rats [21], demonstrating that IL-1β has sexually dimorphic effects on reproductive function. IL-1β of central origin may regulate GnRH neuronal function since central infusion of IL-1β inhibited the secretion of hypothalamic GnRH and plasma LH in a freely moving gonadectomized female rat model [23] while peripheral administration of the cytokine had no effect [4]. Although the in vivo data demonstrate inhibition of GnRH, in vitro studies using the GnRH neuronal GT1-1 cell line or primary hypothalamic culture demonstrated that IL-1β stimulates GnRH release [24, 25], strongly suggesting that the effects observed in vivo that result in inhibition of GnRH secretion may be mediated indirectly via afferent interneurons.

GnRH mRNA Expression Centrally infused IL-1β inhibited the expression of the immediate early genes c-fos and c-Jun during the LH surge in rats as detected by in situ hybridization [3, 26]. A study in castrated males observed no change in GnRH mRNA expression by lateral ventricle infusion of IL-1β into the preoptic area, but IL-1β was found to decrease the translational efficiency of the transcribed mRNA [27]. Posttranscriptional regulation has previously been proposed to play an important role in the production of GnRH [28]. Studies in the female anestrus sheep have demonstrated that IL-1β injected into the third ventricle decreased GnRH mRNA levels in medial preoptic area (MPOA) and in the median eminence [1], indicating that there might be species-specific differences in regulation by IL-1β. In vitro studies have not clarified the effects of IL-1β on GnRH expression. IL-1β receptors have been found in the Gnv-4 GnRH neuronal cell line [29], suggesting GnRH neurons

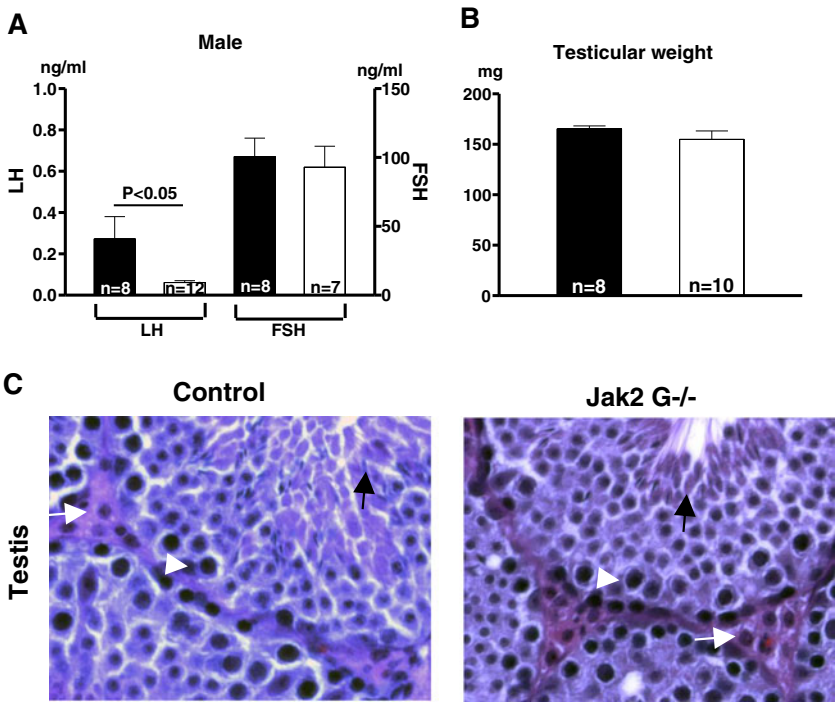

Fig. 3 Male LH, FSH, testes weights, and morphology. **a** Serum LH levels (*left axis*) and FSH levels (*right axis*) for control (*dark bars*) and Jak2 G−/− mice (*open bars*) are displayed, units are nanograms per milliliter. Values are mean± SEM and significant differences are noted with *brackets above bars*. *N*=8 (Control), *N*=12 (Jak2 G−/−). Assay was the same as in [13]. **b** Testis weight of control (*dark bars*) and Jak2 G−/− (*open bars*) mice in milligrams. Values are mean± SEM. *N*=8 (Control), *N*=10 (Jak2 G−/−). **c** Seven-micrometer testis sections with H&E staining. *White arrows* point to Leydig cells, *white arrow heads* point to spermatogonia, and *black arrows* point to spermatids inside of both Control and Jak2 G−/− testis

could directly respond to this cytokine, although this was not directly tested. However, IL-1β did not change the levels of GnRH mRNA in the GT1-1 GnRH-expressing cell line [24, 25].

Interleukin-6 (IL-6) is a 22-kDa cytokine containing 187 amino acids and produced by a variety of cells including astrocytes in the hypothalamus [30]. A recent report observed that the IL-6 receptor α (IL-6Rα) subunit mRNA is expressed in GT1-7 and GN11 cells [31]. IL-6 binds to the IL-6Rα which recruits gp130 homodimerization and activates two main signaling pathways: gp130/Jak/STAT and the SHP2/ERK/MAP kinase cascade [32].

Available data regarding the effects of IL-6 on the reproductive axis remain somewhat controversial. Most in vivo data suggest that IL-6 has no effect on GnRH secretion. For example, the intracerebroventricular administration of IL-6 into the MPOA of ovariectomized female rats had no effect on the release of either GnRH or LH even when infused at supraphysiological concentrations [23]. IL-6 infusion was also shown to have no effects on LH levels in freely moving castrated or intact male rats [21, 33]. Furthermore, no effect on GnRH secretion was observed following IL-6 treatment of hypothalamic explants from proestrus female or male rats [34] although Yamaguchi et al. have noted that IL-6 stimulated GnRH secretion from primary rat hypothalamic cultures in a dose and time-dependent manner [35]. The different results between these groups may be due to the different model (in vivo vs in vitro) and/or different protocols (hypothalamic explants vs primary culture) they applied.

TNFα is a trimeric protein and exists in either a 17-kDa secreted form or in a noncleaved 27-kDa precursor form in

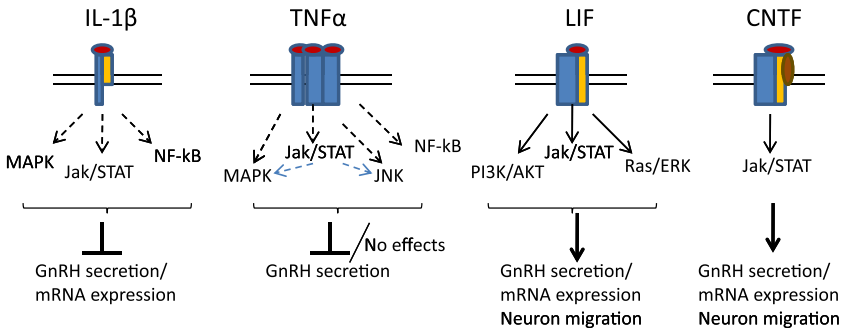

Fig. 4 Signaling pathways of cytokines in GnRH neurons. Il-1, TNFα inhibited GnRH secretion, and/or mRNA expression. LIF and CNTF stimulate GnRH secretion and/or mRNA expression. The *dashed line* representing this signaling pathway is not explored yet, *solid line* representing this signaling cascade has been proved in GnRH neurons, and inverted T represents inhibition; *heavy black arrow* represents stimulation

the cell plasma membrane [36]. It is produced by adipose tissue and released into the circulation [37]. TNFα has been found to exert significant effects on metabolic function in obesity [37] and has been thought to potentially contribute to a link between metabolic status and reproductive function. TNFα exerts its effects via two-cell surface receptors (TNFR1 and TNFR2) that activate a number of cell signaling molecules including Janus kinase (JNK), extracellular-related kinase (ERK) (p44/42), p38 MAP kinase (MAPK), and NF-κB [38], although the effects on P38, MAPK, and JNK may be mediated by TNFR1/Jak2 in various cell types [39].

TNFα receptors are present in the hypothalamus [40]. As noted for IL-6, the effects of TNFα on GnRH neurons are often contradictory. In vivo studies showed that central infusion of TNFα inhibits the GnRH-LH systems [4, 21, 23, 41]. However, in vitro studies have not found an effect of TNFα on GnRH release in hypothalamic explants of male rats or proestrus female rats [34]. LIF is a 20-kDa pleiotropic cytokine containing 181 amino acids. LIF mRNA is expressed in the adult rat brain and other peripheral tissues [42]. LIF exerts its effects through a receptor complex that includes the gp130 subunit but also includes a ligand-specific LIF-specific receptor β (LIFRβ) subunit [43]. Activation of the receptor results in activation of the Jak/STAT signaling cascade or the MAPK/ERK1/2 and PI3K/AKT pathways [44]. LIFRβ and gp130 are both expressed in the GN11 and GT1-7 GnRH neuronal cell lines [31, 45] and LIF was shown to regulate migration of GN11 cells by activating Jak/STAT3, MAPK/ERK1/2, and PI3K/AKT signaling pathways independently [45] and to stimulate GnRH release in GT1-7 cells [31]. Our group has observed that LIF also stimulates GnRH mRNA expression in a dose-dependent manner (unpublished data).

CNTF is a survival factor for various neuronal cell types; it contains 200 amino acids and is 23 kDa in size. It is synthesized by both glia and neurons in the CNS and by Schwann cells in the peripheral nervous system [46, 47]. As for IL-6 and TNFα, the CNTF receptor is a multimer that includes the gp130 subunit, although in this case the receptor is composed of a trimeric complex that also includes the CNTF-Rα and LIF-R subunits [48–50]. CNTF has been shown to maintain motor neuron motility [51]; however, abolition of CNTF expression in mice causes a very mild phenotype with progressive neural motor degeneration later in life coupled with a small reduction in muscle strength [51]. Interestingly, knock out of the CNTF-Rα gene is embryonic lethal [48], suggesting that this receptor subunit may mediate effects of other ligands besides CNTF.

It has been proposed that CNTF exerts facilitatory effects on reproductive function. For example, CNTF stimulates GnRH release in hypothalamic explants from proestrus rats [52] and CNTF blunts the fasting-induced suppression of LH release in rats [21]. Additionally, CNTF increases the amplitude of the LH surge in ovariectomized mice primed with estradiol and progesterone [53]. Dozio et al. observed that the CNTF receptor complex is expressed in GT1-7 cells and CNTF induced GnRH release through a CNTF-CNTFRs-Jak2/STAT3 signaling pathway [54]. They further observed that treatment of GT1-7 cells with 10^{-12} M CNTF resulted in a robust increase of pSTAT3 via activation of JAK2. Our laboratory has found that CNTF increases GnRH mRNA expression in GT1-7 cells via Jak2 using real-time quantitative PCR (unpublished data).

In summary, the cytokine family of hormones interacts with the cytokine class of receptors and has been implicated in the central regulation of reproduction [13]. While we have provided conclusive evidence that cytokine-mediated signaling plays an important role in reproductive development in both males and females ([13] and above), the identity of the specific ligand/receptor interactions that are mediating these effects has not yet been established. Jak is the predominant kinase in mediating the single chain cytokine receptors action and plays an important role in signaling via the gp130 receptor family or the class II cytokine receptors [55]. Although interleukin-1, TNF, LIF, and CNTF are candidates as direct regulators of the GnRH neuron (inhibition or activation) possibly through JAK/STAT signaling pathway (Fig. 4), each of these ligands has been found to activate multiple signaling pathways including the MAPK pathway. Therefore, the Jak2 conditional KO mouse may not fully reveal the significance of cytokine signaling in the GnRH neuron. The precise function remains to be elucidated with the development of additional GnRH neuron-specific receptor knockout models.

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