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Review

# Tachykinins and the hypothalamo–pituitary–gonadal axis: An update

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ABSTRACT

Tachykinins play a critical role in neuroendocrine regulation of reproduction. The best known members of the family are substance P (SP), neurokinin A and neurokinin B. Tachykinins mediate their biological actions through three G protein-coupled receptors, named NK1, NK2, and NK3. SP was suggested to play an important role in the ovulatory process in mammals and humans. Recent findings suggest a role of tachykinins in the aging of the hypothalamo–pituitary–gonadal axis. A high presence of SP was found in the sheep pars tuberalis and evidence indicates that it may have some role in the control of prolactin secretion. The presence of SP was confirmed in Leydig cells of the rat testes of animals submitted to constant light or treated with estrogens. Tachykinins were found to increase the motility of human spermatozoa. Tachykinins were also found to be present in the mouse ovary and more specifically, in the granulose cells. It is possible that tachykinins may play an important role in the ovarian function. NKB has been implicated in the steroid feedback control of GnRH release. Human mutations in the gene encoding this peptide or its receptor (TACR3) lead to a defect in the control of GnRH. A specific subset of neurons in the arcuate nucleus of the hypothalamus, colocalized three neuropeptides, kisspeptin, NKB and dynorphin. This subpopulation of neurons mediates the gonadal hormone feedback control of GnRH secretion. NKB/NK3 signaling plays a role in puberty onset and fertility in humans. This minireview summarizes the recent data about the action of tachykinins on the hypothalamo–pituitary–gonadal axis.



**Contents**



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Tachykinins play an important role in the control of reproductive functions by acting at the hypothalamic, pituitary and gonadal levels (Refs.). Among these peptides, those that have been more intensively studied are substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). They exert their effects by acting on three different types of G protein-coupled receptors named NK1, NK2, and NK3 [65]. Numerous publications and several reviews [16,38,77] reported the effects of tachykinins on reproductive functions and their mechanisms of action. In the past decade, however, the progress on this field, with the exception of NKB, has been rather modest. On the other hand, important new reports have shed considerable light on the role of NKB on the hypothalamo–pituitary–gonadal axis. In the present review, our purpose has been to show the progress made from 1999 up to the present time, on the role of tachykinins in the control of reproductive functions. Some progress has been made on SP, while no significant contributions in this field have been evident with NKA and its two elongated peptides, neuropeptide K and neuropeptide gamma. In contrast to this, the number and importance of reports regarding the role of NKB on the hypothalamo–pituitary–gonadal axis have been nothing short of remarkable. It is therefore inevitable that this review may seem out of balance, as the progress and advances with NKB have considerably outpaced those of SP and the other tachykinins. This review is divided into two sections, the first, dedicated to SP, and the second, to NKB. It mostly covers the reports published during the 2000–2010 decade.

## 1. Substance P (SP)

### 1.1. Studies on primates and humans

Plasma levels of SP were studied during the menstrual cycle in monkeys and humans [43,44]. In the cynomolgus monkey it was observed that plasma SP levels showed significant variations during the menstrual cycle, with higher levels in the follicular phase than in the following luteal period. During the follicular phase there was a negative correlation between plasma levels of estradiol and SP. These results indicate that SP may have a significant role in the events that result in the preovulatory surge of LH and FSH and consequently on ovulation. In normally cycling women during the ovulatory period, plasma SP started increasing during the ascending phase of the LH surge, reaching significantly higher levels during the descending phase of that surge. These changes were parallel to the increasing plasma levels of progesterone during the same period. Taken together, these data suggest the possibility of a significant involvement of SP in the ovulatory process.

### 1.2. Animal studies: SP in the hypothalamo–pituitary axis

In the crested newt *Triturus carnifex*, SP was shown to antagonize the effects of GnRH on the release of gonadotropins [28]. This may be a species-dependent case, as these results, however, are in contrast to what was shown in the case of porcine gonadotropes, where SP demonstrated to stimulate both the release of LH and to potentiate the LH release in response to GnRH. According to the authors [35], this response to SP is dependent on the presence of extracellular $Ca^{2+}$, involving also intracellular $Ca^{2+}$ mobilization. In another study, the concentrations of SP and NKA in the hypothalamus and the anterior pituitary of cyclic, preacyclic and acyclic female rats were compared. In acyclic rats, hypothalamic SP and NKA concentrations were significantly higher than in cyclic and preacyclic rats, but no significant changes were found in the tachykinin concentrations of the anterior pituitary glands of these three groups of rats [21]. In a study carried out in sheep, it was demonstrated that maternal nutrient restriction increased the density and percentage of SP-immunoreactive cells in the anterior pituitary of the fetuses. Likewise, the number of somatotropes that contained SP was also significantly increased in the fetuses from food-restricted mothers [52]. This is a confirmation of previous works demonstrating that many somatotropes in the anterior pituitary gland of different species in addition to growth hormone contain also SP and likely other tachykinins as well. This study demonstrates that the nutritional condition of the sheep has repercussions on the GH-SP presence in the anterior pituitary of the fetus, although these changes seem to be reversible. An interesting finding was reported by Skinner et al. [87], demonstrating the abundant presence of SP in the pars tuberalis of the sheep. This presence is much greater than in the anterior pituitary gland. SP was not present in cells that produce LH in the pars tuberalis. It had been previously hypothesized that the pars tuberalis may secrete a factor, tentatively named "tuberalin", which stimulates prolactin secretion by the lactotrophs in the anterior pituitary gland. Skinner [86] put forward the hypothesis that "tuberalin" may actually be a tachykinin. This hypothesis is of great interest, but only future studies will determine its validity. Yuan et al. [101], reported that in a mouse genetically prone to early senescence by 11 months of age, the concentrations of SP and beta-endorphin in the hypothalamus were significantly lower than in the respective controls. The authors conclude that SP and beta-endorphin may be two of the possible causes of the accelerated senescence of the hypothalamo–pituitary–gonadal axis in these mice. Although this hypothesis is certainly interesting, it is not clear if the low concentration of SP and beta-endorphin in the hypothalamus of the senescence-prone mice may be the cause or simply a consequence of the genetical changes inducing an accelerated senescence. This report does not give any definite clue in this respect. Another problem with this report is that the experiments included only a small number of animals per group, with no subsequent replications.

### 1.3. SP in the testes

In adult rats submitted to constant light, it was demonstrated that, among other markers, in the Leydig cells of the testes, SP was found to be significantly lower in those cells as compared with their respective controls under a normal light–darkness cycle [62]. As in the animals submitted to constant light exposure plasma gonadotropin levels were found to be significantly altered (LH decreased and FSH increased), it is likely that the decrease in the SP expression in the Leydig cells may have been secondarily due to the alterations in the LH secretion. Similarly, in rats treated with estradiol, SP expression in the Leydig cells was also found decreased [63]. These two reports, in addition to confirm the presence of SP in the Leydig cells, also show that the presence of this tachykinin, as well as likely other peptides of the same family, is affected by the alterations of the LH levels in the circulation. These two reports showed a decrease in the presence of SP in the Leydig cells resulting from a decrease of plasma LH, although these results were obtained only through immunocytochemistry. It could have been, however, interesting to determine the actual concentration of this tachykinin by RIA, thus showing the actual concentration in the whole testis, rather than in a histological section. In the Siberian hamster, it was found that Sertoli cells express the preprotachykinin A (Tac1) gene, which suggests the possibility that these cells can also synthesize SP and other tachykinins [17]. Thus, tachykinins may have a modulatory role on the secretory activity of the Sertoli cells. No major seasonal differences were found in the concentrations of tachykinins in the testes of adult rats [96]. In male rats at 21, 31, and 60 years of age, it was reported that there was a significant decrease of testicular SP concentrations during the maturation process. This may have been due to the decreasing proportion of the interstitial sector as the seminiferous tubules increased in

size as soon as spermatogenesis became active after the pubertal stage [97].

SP and NKA have also been demonstrated to increase the motility of human spermatozoa, this action was concentration-dependent. The specificity of this effect was certified by the fact that the incubation with NK1 and NK2-receptor blockers nullified these effects [78]. Furthermore, human spermatozoa expressed the NK1 and NK2 receptors and also expressed Tac1 gene, which encodes the synthesis of SP and NKA. These results are very interesting, as they show that tachykinins may likely play a role in the motility of spermatozoa, which eventually may result in an effective trajectory in the female reproductive system in order to achieve fertilization.

### 1.4. SP in the ovary

Tachykinins, such as SP and NKA, have also been found in the mouse ovary, and their concentrations were decreased by the treatment with gonadotropins [13,14]. It is possible that SP and other tachykinins may have a modulatory function on the ovary. In the adult rat between 5 and 25 months of age, no significant changes were found in the concentrations of SP and NKA in the ovary. Only in 25-month old rats SP concentrations were found to be higher than at earlier ages [22]. Expression of the genes that encode the synthesis of SP and NKA was found in the granulose cells of the mouse [69]. NK1 and NK2 receptors were also found in these cells. Mouse oocytes expressed the same genes and the receptors [69]. It can be speculated that tachykinins may be important in the mouse oocyte to stimulate smooth muscle contraction in the Fallopian tubes and also to facilitate implantation. Capsaicin, a drug that induces release and depletion of tachykinins, when administered neonatally into mice and rats resulted in a reduced fertility [69].

### 1.5. SP in other reproductive organs

In the mouse uterus, the expression of Tac1 and Tac2 and tachykinin receptors was demonstrated. Ovarian steroids were shown to regulate the expression of tachykinins and their receptors in the mouse uterus [64,70]. The presence of SP was also revealed in the human placenta, a tissue capable to produce hCG and sex steroids [53]. By immunocytochemistry, SP was localized in the trophoblast (syncytium and cytotrophoblast) of the chorionic villi. However, SP was not shown to be able to modify the secretion of hCG and progesterone when tested in vitro. It is speculated that SP may be a regulator of the placental circulation.

It was previously shown that SP is present in the pineal gland, which in turn can modulate pituitary secretion of different hormones [15,56,72], such as the gonadotropins. It was recently demonstrated that SP can modulate melatonin secretion by the pineal gland, therefore potentially being able to influence gonadotropin secretion [57].

### 1.6. Conclusions

The reports on SP here reviewed in general confirm and further expand our knowledge on the role of SP in the hypothalamo–pituitary–gonadal axis. Very few breakthroughs in this field, however, have been reported. Perhaps one of the most potentially important is the demonstration of high levels of SP in the pars tuberalis. It remains to be demonstrated if SP could be a good candidate for a "tuberalin" factor, which could be involved in the control of prolactin secretion. Another potentially important finding is that SP may be involved in the senescence of the hypothalamo–pituitary–gonadal axis, although the evidence provided so far in this respect is far from decisive and further studies are needed to certify this involvement. Finally, it was confirmed that SP is present in the gonads, where it may exert paracrine actions on the ovarian and testicular functions.

## 2. Neurokinin B

In the past decade remarkable progress has been made regarding NKB and its role on the reproductive functions. Different methodologies have greatly contributed to our understanding of the role of this tachykinin on the hypothalamo–pituitary–gonadal axis. It has become evident that NKB fulfills a very critical role on this axis, and its main actions seem to be exerted at the hypothalamic level. Laboratory animals, receptor biology, human subjects, and molecular biology studies have all contributed to the decisive progress made in this field. In particular, reports studying defects involving NKB-related genes in humans and the repercussions that these defects have in the reproductive functions have been particularly enlightening. The purpose of this part of the present review has been to show how all these numerous reports have contributed to a better knowledge of NKB and its influence on the reproductive functions.

NKB is predominantly expressed in the arcuate nucleus (ARC) of the hypothalamus [18,31,81] and NKB signaling has emerged as a key player in the neuroendocrine regulation of reproduction [76].

NKB has been implicated in the steroid feedback control of GnRH release [77]. It has been very recently discovered that human mutations in the gene encoding this peptide (called TAC3) or its receptor (TACR3) lead to a defect in the control of GnRH with subsequent hypogonadism [32,94]. It was also recently reported that a subset of neurons in the arcuate nucleus (ARC) of the hypothalamus colocalized three neuropeptides, kisspeptin, NKB and dynorphin (Dyn) [23,30,51]. Each of these neuropeptides has been shown to play a critical role in the central control of reproduction both in normal physiological condition and in reproductive disorders.

### 2.1. NKB and tachykinin receptors

The neurokinin-3 receptor (NK3R) is the tachykinin receptor that binds NKB with highest affinity among the known tackykinins [45].

The NK3R belongs to the family of G-protein coupled receptors [65] that after activation are internalized into endosomes [20]. Internalization of NK3R in the supraoptic nucleus was observed after injection of a NK3 agonist implying receptor activation [33,36]. On the other hand, NK3-ir cells are distributed in areas like the POA and the ARC in the rat [54], guinea pig [100], sheep [2] and human brain [54], all areas involved in the control of reproductive neuroendocrine function

### 2.2. Effect of NKB or its agonists on gonadotropin secretion

Administration of NK3R agonists has shown variable effects on LH secretion depending on the animal model and the steroid milieu. NKB appears to be inhibitory in rodents since senktide, a NK3R agonist, suppressed LH secretion in rats and mice [39,58,81]. The inhibitory effect on LH release was also observed in goats [98]. In male rodents, NKB injected ip or icv had no effect on LH circulating levels and failed to stimulate GnRH secretion in hypothalamic explants [10]. On the contrary, senktide, injected intraventricularly, stimulated LH secretion in the ewe [3] and produced an increase in LH levels similar to those found in the preovulatory LH surge. In the presence of physiological levels of E2, senktide increased LH levels in female rats [59]. Both NKB and senktide stimulated LH levels in prepuberal rhesus monkeys after i.v. administration [74]. NKB may stimulate LH release in humans since mutations in NK3R gene are

associated with infertility in humans [94]. Therefore, it seems that the effects of NKB may be species-dependent.

### 2.3. NKB in the hypothalamus

A recent study indicates that NKB, kisspeptin and Dyn are localized in a subpopulation of neurons in the human ARC nucleus [76] as it has been previously demonstrated in other species [23,30]. Then, the three neuropeptides are produced by a single population of cells in the ARC and all three have been independently linked to GnRH secretion.

NKB neurons in the ARC of the rat, sheep, mouse and goat coexpress Dyn [4,23,58,98].

Dyn, acting via the K opiod receptor, inhibits LH release [46,84]. On the other hand, kisspeptin, which plays a critical role in the neuroendocrine regulation of reproduction [71] activates GnRH neurons [60]. Moreover, pulsatile secretion of kisspeptin and GnRH are temporally linked [47,50].

It is not clear whether the modulation of NKB on LH secretion is mediated directly on GnRH neurons, which are the final common pathway by which the brain regulates gonadotropin secretion.

Although direct contacts are present between NKB fibers and ovine GnRH cells [31] and NK3Rs are expressed in GnRH neurons of the mice [93], only few GnRH neurons in the rat contain immunoreactive NK3R [48] and GnRH neurons in the ewe lack NK3R [2], indicating the possible involvement of interneurons in mediating the influence of NKB onto GnRH neuron.

Recent data indicate that NKB/Dyn/kisspeptin neurons provide direct inputs to GnRH neurons in the POA as well as the MBH at the level of cell bodies and dendrites in the rat [9,47], mouse [5] rhesus monkey [73], sheep [51] and human [11]. These neurons also project to the median eminence establishing close contacts with GnRH terminals in the external zone in rodents, monkeys and sheep [9,49,73]. Finally, GnRH neurons express both kisspeptin (Kiss1r) and NK3 receptors [37,48]. However, the presence of few NK3R in GnRH neurons suggests that the actions of NKB on GnRH neurosecretory activity might be mediated indirectly via other neurons and/or neuropeptides. Moreover, kisspeptin acts at both the median eminence [12] and GnRH cells bodies in the POA [66,67], but in this case again, the possibility that interneurons play a role in the control of GnRH release cannot be excluded [23,30].

Colocalization of NK3R in NKB neurons of the ARC suggests a potential mechanism for the autoregulation of this subpopulation. Several reports showed colocalization of NK3R in NKB neurons [4,23,58,76,98]. In rat and sheep, the majority of NKB cells in the ARC colocalized with NK3R [1,4]. Close contacts are frequent between NKB terminals and NKB/NK3R cells in the ARC. This suggests a target for reciprocal interactions among these cell populations of NKB/Dyn/kisspeptin neurons in the ARC, which convey a stimulatory effect to GnRH neurons via release of kisspeptin. Then, kisspeptin, Dyn and NKB are all available to act as co-transmitters or neuromodulators at the targets of projections of kisspeptin/Dyn/NKB soma in the ARC, being these cells themselves the targets of these peptides. In summary, the anatomical evidence suggests that NKB/Dyn/kisspeptin neurons in the ARC comprise an oscillatory feedback loop interconnected through collaterals that synchronize their activity.

### 2.4. NKB and the pulsatile secretion of GnRH

Studies using electrophysiological techniques to record multiple-unit activity (MUA) indicate that periodic bursts (volleys) are associated with LH pulses and by inference also with GnRH [61]. NKB induces MUA volleys suggesting that NKB expressed in kisspeptin neurons in the ARC are involved in the process of generating the rhythmic discharge of kisspeptin that drives pulsatile GnRH and LH secretion and this is influenced by the sex steroid milieu [98].

Only high affinity receptor for NKB (NK3R) and k-EOP receptor (KOR), the opiod receptor subtype with highest affinity for Dyn is expressed in NKB/Dyn/kisspeptin neurons [2,48,58]. On the contrary, kisspeptin receptor (Kiss1r) is not expressed in kisspeptin neurons suggesting no localization in these neurons [4,34]. GnRH fibers and terminals do not express KOR [55,83] suggesting that GnRH fibers are sites of action of kisspeptin and NKB but not of dynorphin.

Navarro et al. [58] proposed that NKB could limit the release of GnRH acting via NK3 on GnRH fibers. In addition to activating kisspeptin neurons, NKB also acts on NKB receptors on GnRH nerve terminals in the median eminence. While kisspeptin activates GnRH terminals, NKB could be acting through a slower Gi-coupled NK3 pathway, which would attenuate or diminish the response to kisspeptin. The inhibitory signaling could be also accomplished by some other neuropeptide or co-transmitter.

### 2.5. NKB and the steroid feedback

NKB/Dyn/kisspeptin neurons in the ARC have been proposed to be primary site of negative feedback action of E2 and progesterone (P) on GnRH secretion [4,23,30,88–90,98]. During the female reproductive cycle, the neuroendocrine action of estradiol (E2) switches from negative feedback to positive feedback to initiate the preovulatory GnRH and subsequent LH surges. Estrogen receptor-α (ERα) is required for both E2 negative and positive feedback regulation of LH.

There is a high degree of colocalization of NKB/Dyn/kisspeptin neurons with gonadal hormone steroid receptors including ERα [4,25,31,88], progesterone receptors [24] and the androgen receptor [6,7,8,88].

In the ARC, NKB/Dyn/kisspeptin cells, which stimulate basal GnRH/LH release, are negatively regulated by E2. When E2 levels decline, kisspeptin/Dyn/NKB neurons became spontaneously active and this activity could be amplified by positive autosynaptic feedback through NKB/NK3 signaling [58]. Moreover, when E2 levels are low, Dyn, KOR and NK3R expression increases in the ARC as well as NKB and kisspeptin expression [18,19,75,89] so Dyn and NKB and their receptors are targets of E2 regulation.

On the other hand, rising levels of E2 would reduce the stimulatory drive of NKB/NK3 signaling in the ARC NKB/Dyn/kisspeptin neurons by suppressing the expression of NKB [18,58,75] as well as NK3 [58], which then would reduce the periodic burst associated with LH pulses. The cytosolic distribution of NK3R increased in the ARC during the follicular phase in association with alterations of the steroid milieu [1].

E2 inhibits the expression of NKB and NK3R mRNA in the ARC and the effect is greater in the afternoon of proestrus when circulating levels of E2 are high [59] indicating that NKB/NK3R signaling in NKB/Dyn/kisspeptin neurons in the ARC is regulated by an E2-dependent negative feedback.

Kisspeptin release by NKB/Dyn/kisspeptin neurons in the ARC mediates the negative feedback actions of E2 as well [60]. E2 increases Kiss1-expressing neurons in the ARC in mice [60,88,92] sheep [90] primates and in postmenopausal women [79]. E2 inhibits GnRH/LH pulse amplitude by suppressing kisspeptin release from NKB/Dyn/kisspeptin neurons during breeding season [91] On the other hand, Dyn mediates progesterone negative feedback in ewes and primates [51]. Since Dyn inhibits MUA volleys and the central administration of a KOR antagonist reverses the inhibitory effect of progesterone on pulsatile LH secretion [29], Dyn/KOR signaling could also mediate the negative feedback action of progesterone [98].

Estrogen receptor alpha (ERα) is present in a large percentage of NKB neurons in the ARC of the ovine hypothalamus [31]. In rats [75] and monkeys [82] the expression of the preprotachykinin B gene is increased after ovariectomy, and estradiol replacement decreases preprotachykinin B mRNA in ovx rats [75] and NKB expression in the infundibular nucleus in the ovx sheep [68]. NKB mRNA in the ARC increases following gonadectomy, [18,80,82] in conjunction with a rise in LH secretion, suggesting that NKB signaling is stimulatory to the reproductive axis.

The role of NKB/Dyn/kisspeptin neurons in the E2 positive feedback depends on the species studied [51]. In contrast to other mammals, NKB/Dyn/kisspeptin neurons do not play a role in LH surge in rodents. On the other hand, the evidence is strong in the ewe since in the pre-ovulatory surge, E2 induces the release of kisspeptin and NKB, but this evidence is only indirect in primates. However, available data do not indicate which neurotransmitter is involved, even though kisspeptin is the obvious candidate and the second one is NKB [51].

### 2.6. NKB gene studies in humans

As we mentioned before, disabling mutations of either NKB (known as TAC3 in humans) or the NK3 receptor (known as TACR3 in humans) are associated with reproductive failure [94] indicating the probable stimulatory role of NKB on GnRH release. The fact that TAC3 and TACR3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans indicates that both NKB and NK3R play a crucial role in hypothalamic GnRH release in humans [99]. Recent reports of humans who have normosmic idiopathic hypogonadotropic hypogonadism due to TAC3 and TACR3 mutations provide evidence for the involvement of NKB signaling in puberty onset [26,95]. Indeed, humans lacking NKB/NK3 signaling have delayed the onset of puberty and impaired fertility [85,94]. It has been recently demonstrated that gonadal hormone-independent central restraint on pubertal timing in mice involves NKB/kisspeptin neurons in the ARC [40–42] These authors found sex differences in the regulation of LH secretion and Kiss1 and NKB expression in the ARC of juvenile mice; these differences may contribute to the differential timing of puberty onset between the sexes.

It has been recently described that post menopausal hypertrophied neurons expressed kisspeptin, NKB, substance P and ERα mRNA [76] Since ovariectomy in experimental animals indicates similar findings [75,81,89,90] these changes may be a compensatory response to ovarian failures. Thus, NKB plays a role in the reproductive neuroendocrine regulation both in puberty and menopause.

In Fig. 1, we summarized a very simplified model proposed for the role of kisspeptin/NKB/neurons in the release of GnRH. NKB/Dyn/kisspeptin neurons in the ARC send projections to GnRH terminals and give off collaterals to neighbor neurons and themselves in the ARC. Hypothetically when NKB overcomes the inhibition provided by Dyn, these neurons begin to release kisspeptin and then GnRH is released in a pulsatile fashion. The possibility that interneurons may be involved in the effect of both kisspeptin and NKB on GnRH secretion cannot be ruled out.

### 2.7. NKB in the gonads

NKB, as previously described for SP and NKA, stimulated the motility of human spermatozoa and this effect was antagonized by a NK3 receptor blocker [78]. Also, the presence of NK3 receptors and expression of Tac3, which encodes the synthesis of NKB, was found in human spermatozoa. The highest presence of NK3 receptors was found in the midpiece of the spermatozoa [78].

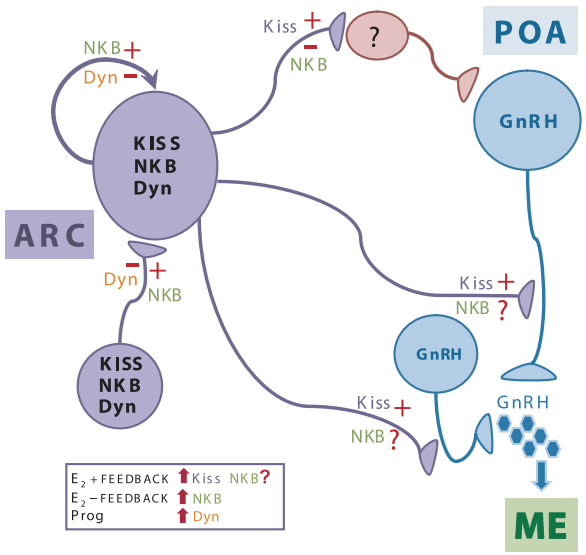


**Fig. 1.** Schematic representation of the NKB neurons that coexpress kisspeptin and Dyn in the ARC and their hypothetic role in GnRH secretion in the ME. When E2 levels are low, NKB/Dyn/kisspeptin neurons become active and this activity could be amplified by positive autosynaptic feedback through NKB/NK3 signaling. When E2 levels rise, the stimulatory drive of NKB/NK3 signaling in the NKB/Dyn/kisspeptin neurons could diminish by suppressing the expression of NKB and NK3R and KOR that would result in a stimulatory effect to GnRH neurons via release of kisspeptin. On the other hand, Dyn mediates progesterone negative feedback on GnRH release. Besides, NKB release from ARC could also be acting directly on GnRH neurons in the POA or MBH or indirectly on interneurons.

In the rat ovary, it has been described that Tac2 expression became evident at 4 weeks of age, while Tac1 expression was not detected before 6 months of age [27]. In hypothyroid rats, ovarian Tac2 expression was found to be decreased. Interestingly, when cyclicity ceased in old rats, ovarian Tac2 expression decreased and concomitantly ovarian Tac2 expression also decreased. These findings suggest the possibility that tachykinins may be involved in the senescence of the rat ovary. It is still unclear, however, if ovarian NKB is the cause or the consequence of the aging process on the ovarian function. Thyroid hormones seem to regulate tachykinin expression in the rat ovary [27].

In summary, the available data summarized here, indicate that NKB works in concert with kisspeptin and Dyn to control pulsatile GnRH release and GnRH plays a important role in sex–steroid feedback on gonadotropin secretion.

### 2.8. Conclusions

Compelling evidence suggests that NKB has an important role in the regulation of GnRH release and therefore on the reproductive functions. The fact that humans with mutations in the gene encoding either NKB or its preferential receptor have hypogonadism and delayed puberty suggests a stimulatory influence of NKB on the reproductive system. The administration of NKB or some of its agonists produced, however, some conflicting results, which may be due to species differences in the NKB effects. Direct connections between NKB and GnRH neurons also point to a critical influence of NKB on GnRH release. Furthermore, NKB neurons in the hypothalamus contain steroid receptors, and therefore this indicates that these neurons are involved in the steroid feedback that controls gonadotropin secretion.

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