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GnRH Effects Outside the Hypothalamo-Pituitary-Reproductive Axis

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Abstract

GnRH is a hypothalamic decapeptide with an undisputed role as a primary regulator of gonadal function. It exerts this regulation by controlling the release of gonadotropins. However, it is becoming apparent that GnRH may have a variety of other vital roles in normal physiology. Reconsideration of the potential widespread action that this traditional reproductive hormone exerts may lead to the generation of novel therapies and provide insight into seemingly incongruent outcomes from current treatments using GnRH analogues to combat diseases such as prostate cancer.

Keywords

hippocampus; olfactory system; heart; bladder; somatotropes

Introduction

Gonadotropin-releasing hormone (GnRH) was among an array of hypothalamic releasing factors discovered nearly four decades ago by the laboratories of Schally and Guillemin (1). Confirmation that GnRH was released into hypophyseal portal blood (2,3) cemented the contention that this decapeptide was unique to the reproductive hypothalamo-pituitary axis. However, there are occasional reports that GnRH has unexpected effects or is present in non-reproductive tissues forcing us to reconsider this restricted reproduction-only view. For example, GnRH has activity on the sympathetic ganglia of the frog (4), GnRH receptor expression is present in the cerebellum (5) and bladder (6), and GnRH is released in significant concentrations into cerebrospinal fluid (7), to potentially act outside the hypothalamus through volume transmission (8). Indeed, GnRH may have evolved with functions extraneous to reproduction. Studies on octopi provide evidence that GnRH has potent cardiovascular roles (9).

More than 40 different GnRH precursors have been identified (10,11). Most evidence in mammals indicates that GnRH I and chicken GnRH II have been conserved in this Class although GnRH II has not been retained in all species (12). In mammals, two GnRH receptors have been identified, type I and type II, but the type II GnRH receptor has been

silenced in several species (10,12). It is possible that the functions of GnRH II and the type II GnRH receptor have been assumed by GnRH I and/or the type I GnRH receptor. Except for an evolutionary context, this review will focus on GnRH I and the type I GnRH receptor.

There is compelling evidence that GnRH and its receptor may perform fundamental roles in cancer cells (see (13) for recent review) but it is arguable that these tumor effects do not occur in “normal” physiology. There are also several studies showing local GnRH and GnRH receptor production in extra-pituitary reproductive tissues: endometrium (14), ovary (15,16), placenta (17-19), testis (20,21), prostate (22). Thus, the purpose of this short review is to summarize evidence that, in addition to its well-established reproductive roles, GnRH may affect multiple tissues not directly associated with the reproductive axis or cancer. Table 1 summarizes putative non-reproductive sites of action of GnRH in mammals. This review will address areas in which studies have attempted to address the physiological significance of GnRH effects.

GnRH influence in the central nervous system

It has been known for nearly 3 decades that GnRH can affect the central nervous system. Not only can GnRH depolarize sympathetic ganglion neurons in the frog (4,23,24) but, in the rat, GnRH has been shown to affect hypothalamic (25,26), hippocampal (27-29), cerebellar (30), preoptic (31,32) and cortical (30,33) neurons. Indeed, there is evidence that GnRH may influence neurons in numerous other locations (34-39). Although GnRH projections may be widespread in the brain (40-44), the discovery that GnRH is released by the median eminence in large quantities into third ventricle cerebrospinal fluid (Figure 1A; (7,45-48)) frees this decapeptide from the constraints of synaptic transmission expanding its potential sphere of influence. As noted in Table 1, several areas within the central nervous system have been reported to express GnRH receptors.

Hippocampus

The hippocampus consistently expresses high levels of GnRH receptors. In the human hippocampus, pyramidal neurons were also recently found to express immunoreactive GnRH receptors (49). Similarly, GnRH receptor-immunoreactive neurons were found almost exclusively within the pyramidal cell layer, dentate gyrus, and indusium griseum of the mouse and sheep (Figure 1B; (39)). Hippocampal pyramidal neurons in the rat take up I^{125} -buserlin when it is injected into the lateral ventricle (37) but it should be noted that endogenous GnRH is not detectable in the lateral ventricle (48). There is evidence from the rat that the indusium griseum receives GnRH projections (40) and GnRH has been detected in human hippocampus extractions (50). GnRH alters the electrical properties of rat hippocampal pyramidal cells (27-29) and stimulates increased IP_3 production within these cells (51). Both these effects are modified by estrogen in the rat (27). In sheep, hippocampal GnRH receptor-expressing neurons co-express $ER\beta$ (39). As GnRH is likely to be elevated post-menopause due to the loss of estrogen negative feedback (52), the effect of GnRH on these neurons may constitute a component of the neurodegenerative pathology that accompanies Alzheimer's disease (53). It is notable that hippocampal spinophilin, a reliable dendritic spine marker, is significantly decreased in response to high doses of GnRH (54).

Olfactory system

GnRH receptor expressing neurons are evident throughout the olfactory system in the rodent (36,39,55). These structures include the mitral cell layers of the olfactory and accessory olfactory bulbs, piriform cortex, tenia tecta and amygdala. GnRH has been detected in the hamster accessory olfactory bulb (42) and in the rat piriform cortex (55). The tenia tecta contain a discrete population of testosterone sensitive GnRH-immunoreactive neurons in the

hamster (43,56). GnRH has also been reported within the terminal nerve, which projects to several olfactory regions (57) and is a structure associated with reproductive behavior in hamsters (58,59). It is noteworthy that olfactory bulbectomy eliminates mating behavior in hamsters (60), mice (61) and shrews (62). GnRH has been proposed to alter the detection of specific odors relevant to reproduction via a neuromodulatory effect (57). Such modulation may be the cause of variations in smell perception across the menstrual cycle (63,64). GnRH receptor-expressing neurons were distributed throughout the amygdala (39). Although some (37) have reported a limited distribution of GnRH binding sites in the rat amygdala, others have detected a high density of potential GnRH receptor expressing neurons in this region of the mouse (34) and rat (65). GnRH may access these receptors through neurons which project directly to the amygdala (66). Lesions of the amygdala decrease lordotic behavior in the rat (67) and prevent ovulation (66).

Central grey and sexual behavior sites

GnRH receptors have been reported within the central gray of the rat (37), mouse (39) and sheep (39). Importantly, GnRH injections into the rat central gray potentiated lordosis (68,69). GnRH immunoreactive fibers have been identified within the rat central gray (41) and significant amounts of GnRH have been extracted from midbrain preparations in this species (70). Additionally, the intimate association between the central gray, the 4th ventricle and cerebral aqueduct allows potential access for CSF-borne GnRH. We have shown that, in sheep, during the LH surge GnRH concentrations will be elevated in this vicinity (48).

Cerebral cortex

GnRH binds to cerebral cortex neurons (37), which express immunoreactive GnRH receptors (39,71). Indeed, GnRH receptor-immunoreactive neurons in the cerebral cortex are widespread, suggesting that GnRH may act as common neuromodulatory peptide. In the rat, GnRH depresses the activity of cortical neurons (30,33) and has been shown to affect neurite outgrowth and neurofilament protein expression in cultured cortical neurons (72). We are unaware of GnRH immunoreactive fibers being reported in cortical regions. We have already noted the presence of GnRH in the piriform cortex (55). Low levels of GnRH have been reported in the human cortex (50). In addition, the splicing intermediate of mature GnRH mRNA, which still contains intron A, has also been detected in the rat cortex (73,74). It is possible that third ventricle CSF-GnRH accesses these cortical GnRH receptor-expressing neurons, especially during the GnRH surge as this lasts for over 40 hours. In support of this conjecture, Chauhan et al (75) injected trypan blue into the third ventricle of the mouse and, after 24 hours, this trypan blue was detected in the dorsal cortex. Similarly, when the 40kDa plant glycoprotein, horseradish peroxidase, is injected into the lateral ventricle, it distributes widely and is evident in cortical areas within 4h (76). There is evidence in women that chronic GnRH agonist administration affects cortical functioning (77,78) but these studies cannot discriminate between direct GnRH effects or the induced hypoestrogenic environment. However, it is noteworthy that exogenous GnRH can access the brain (48).

Lateral septum, preoptic area and arcuate nucleus

GnRH receptor-expressing neurons in the lateral septum (34,39) provide neuroanatomical support for why GnRH administered to this region affects thermoregulatory activity in the rat (79,80). It is noteworthy that dysregulation of the GnRH system has been suggested as a causative factor in hot flashes (80,81). However, in a preliminary study in sheep (Figure 1C), we found no effect of 1mg GnRH i.v. on peripheral thermoregulatory events. This 1mg dose elevates CSF-GnRH into the physiological range (48). The presence of GnRH receptor-expressing neurons in the preoptic area and arcuate nucleus (39,82) is in keeping with the findings of electrophysiological studies (25,26,31,32). As these regions have a surfeit of

GnRH in their vicinity, the potential source of ligand is not a conundrum. It has been hypothesized that GnRH may modulate its own release through an ultrashortloop feedback system (83,84). This hypothesis is supported by evidence that some GnRH neurons are electrophysiologically responsive to GnRH (31,32) and also express GnRH receptors (85). However, we found that physiological infusions of exogenous GnRH into the third ventricle did not perturb endogenous GnRH release (86). GnRH neurons receive input from far more neurons than previously thought (87) and thus it is possible that any effects of this exogenous GnRH (86) on endogenous GnRH release may have been countered by input from these neurons.

Cerebellum and motor control sites

GnRH receptor expression within the superior colliculus (39), red nucleus (39) and cerebellum (5,88,89) suggests that GnRH may modulate motor control. Previous studies have reported GnRH binding within the superior colliculus (90). The red nucleus has been implicated in movement, possesses cerebellar connections, and projects to the olivary nucleus (91,92). It is noteworthy that the red nucleus contains an abundance of dopamine neurons and low levels of immunoreactive GnRH have been reported in the human red nucleus (50). As GnRH inhibits the synthesis of dopamine (93), it may act within the red nucleus to regulate dopamine production. The cerebellum plays complex roles in motor behavior and cognition. Cerebellar Purkinje cells are GABAergic and provide inhibitory output from the cerebellum while cerebellar granule cells act to modulate the actions of the Purkinje cells through excitatory glutamatergic input (94). Centrally administered GnRH significantly affects both cerebellar glutamate and GABA content (95). The source of GnRH for these cerebellar and other motor control sites is unclear but GnRH immunoreactivity has been reported in Purkinje cells (89) and low levels of GnRH have been detected in extracts of the middle lobe of the human cerebellum (50). There is also evidence that antibodies administered into the third ventricle have access to the cerebellum (75). Thus, CSF-GnRH may affect this part of the brain.

Cerebellar GnRH activity may provide a correlative link between seemingly different symptoms associated with at least two genetic disorders, Gordon Holmes' syndrome (GHS) and Boucher-Neuhauser syndrome. GHS is characterized by cerebellar ataxia and gonadal insufficiency. The gonadotropin deficiency is not reversed with GnRH treatment suggesting gonadotrope insensitivity (96). GHS is attributed to an autosomal recessive genetic defect (97) but there is no evidence that the GnRH receptor gene is mutated (96). This does not eliminate problems with GnRH and its receptor as potential key players in GHS pathology as mutation of possible downstream targets that would cause problems with activation of the receptor such as G-protein coupling, glycosylation, or second messenger systems. Boucher-Neuhauser syndrome is characterized by the same symptoms as GHS but with the addition of chorioretinal atrophy (98). GnRH and the GnRH receptor have been reported in the retina of mammals (88,99) and fish (100); GnRH may play a role in normal ocular development in the zebrafish (101). Testing the hypothesis that GnRH has a physiological role in the mammalian cerebellum may be technologically challenging. It is of interest that Minakata et al (102) reported in this issue that administering GnRH into the octopus cerebellum has profound effects on motor activity, providing some preliminary support for this hypothesis.

Thus, there is compelling evidence that GnRH may act on several sites throughout the brain. However, apart from strong evidence that GnRH plays a role in sexual behavior, unequivocal data supporting physiologically functional roles for these other GnRH receptor expressing sites in the mammalian central nervous system is lacking.

GnRH affects pituitary cells other than gonadotropes

Hypothalamic factors, although named according to their first discovered function, are known to stimulate the release of pituitary hormones not associated with their name. For example, thyrotropin-releasing hormone has been shown to stimulate growth hormone (GH) and prolactin release (103,104). On the other hand, the recently discovered prolactin-releasing factor has no effect on prolactin release *in vivo* (105,106). GnRH has been shown to stimulate prolactin release in the rat (107) but this effect is thought to be mediated through paracrine modulation of lactotropes by gonadotropes (108). It is notable that in mammals (rat (109), mouse (110), monkey (111), sheep (112)), a proportion of gonadotropes express GH (or somatotropes express LH). We (113) and others (114,115) have also observed in sheep that at the time of the estradiol-induced LH surge there is a concomitant GH surge. In fish, GnRH is a potent stimulator of GH release (116) and a significant proportion of chicken somatotropes respond to GnRH (117). Villalobos et al (118) showed that all cell types (GH, ACTH, TSH, prolactin) in the rat pituitary responded to GnRH with both an increase in intracellular Ca^{2+} and in hormone release. Although it is maintained that, in higher vertebrates, GnRH does not stimulate GH release (119), there have been few studies. GnRH-induced GH release has been reported in some (120), but not all (121), normal males, many studies have observed an effect of GnRH on GH release in persons with disorders: anorexia (122); schizophrenia (123); acromegaly (124); diabetes (125); Klinefelter's syndrome (126). GnRH-induced GH secretion was observed *in vitro* in the rat, but only in the early post-natal period (107,127). Our preliminary studies in ovariectomized ewes (Figure 1D) suggest that a physiological dose of GnRH is able to elicit an increase in GH release. One putative role of this GnRH-induced GH secretion may be in luteogenesis following the preovulatory LH surge. LH and GH are the primary luteotropic hormones, which support the development and function of the corpus luteum in domestic ruminants (128).

Taken together, these studies suggest that although GnRH plays a fundamental role in pituitary gonadotropin regulation, GnRH may also affect the secretion of other pituitary hormones. The relative physiological importance of these non-gonadotropic effects may be species dependent. Thus, in lower vertebrates and mammals, GnRH-induced GH secretion may be critical whereas in others, such as humans, these effects may have become vestigial and only invoked during disease.

GnRH effects outside the pituitary and brain

GnRH binding has been detected in multiple extra-CNS sites (19,129) but only the presence of GnRH receptors on tumors has attracted considerable attention due to the therapeutic potential of co-opting their use to target the delivery of toxic substances to cancer cells (130,131). Apart from the noted reproductive tissues that express GnRH receptors (14-18,20-22), there are several other sites expressing GnRH receptors warranting significant further study.

Heart

It is noteworthy that the presence of GnRH and GnRH receptors in the heart of lower vertebrates, especially fish, is well established (132-137). In an elegant study knocking down GnRH by blocking GnRH mRNA translation, cardiac development in the zebrafish was significantly impaired (138). Studies injecting biotinylated GnRH (89), radioactive GnRH (139,140) or GnRH agonists (141-143) have consistently reported GnRH binding in the rodent heart. GnRH receptor mRNA has also been detected in the human heart (19). Immunoreactive GnRH receptors have also been noted in the human heart, with highest GnRH receptor levels evident in the infarcted heart (144). Moreover, for cetrorelix, a GnRH

agonist, the total amount of cetrorelix bound to the rat heart was nearly 50% of the amount bound to the pituitary gland (145). GnRH has been reported in the rat heart (89,146,147) and GnRH mRNA is measurable in the human (19) and mouse (148) heart.

Men who are chemically castrated are at a significantly increased risk of a serious cardiovascular event (149,150). This may be due to the loss of testicular androgens: androgens are known to affect cardiac contractility (151,152) and low circulating androgen levels are linked to cardiovascular disease (153). However, a recent epidemiological study on 73000 men, which compared chemically *vs* surgically castrated men, strongly supports the hypothesis that the increased risk of cardiovascular disease is not due to the loss of androgens (154). Subsequent studies (155-158) have confirmed this seminal investigation. Our preliminary *in vitro* investigations demonstrate a direct effect of GnRH, as low as 1pg/ml, on the contractility of murine cardiomyocytes in serum-free, and thus androgen-free, media (159). Studies in the chemically castrated rat using the GnRH agonist, Zoladex, suggest this may translate into impaired cardiac function *in vivo* (160,161). It was not established whether this impaired cardiac function was due to the GnRH agonist *per se* rather than the loss of testosterone.

Adrenal

In several species, including humans, binding of GnRH analogs or GnRH receptor mRNA have been reported in the mammalian adrenal (17,142,145,162-164). Administration of a GnRH analogue, Surfagon, induced morphological changes in the adrenal cortex of the male mouse and, importantly, these effects persisted in castrated animals (165). In castrate or ovariectomized ferrets, the GnRH agonist deslorelin significantly improved adrenocortical disease (166). Treating female rats for 3 months with the GnRH antagonist, Detirelix, caused a significant reduction in the adrenal/body weight ratio (167). However, whether these effects are directly on the adrenal gland or indirectly through modulation of gonadotropin release has not been established.

Bladder

The human bladder epithelium produces both GnRH and GnRH receptor (6). Following ³H-GnRH injection significant accumulation of radioactivity was reported in the mouse bladder (139). The putative function of this paracrine GnRH system has been investigated in the dog (168-172). Ovariectomy causes incontinence in dogs. Treatment with the GnRH agonist, deslorelin, restored continence to all ovariectomized incontinent animals (169). With the loss of ovarian steroids and an absence of a relationship between gonadotropin levels and urodynamic function, the effect was considered directly due to GnRH (168) and confirmation of the GnRH receptor in the bladder of this species (171,172) supports this hypothesis. It is not known how GnRH affects bladder function but urethral closure pressure is unaffected by GnRH (170).

There are other GnRH target sites that have received scant attention to date, such as the skin (142,173), lymphocytes (174), kidney (131,140,142,175) and liver (140,142,175). Several studies have shown that GnRH binding may occur in the liver and kidney (142,175) but discussion of these data has argued that these sites are involved in peptide degradation, despite evidence that GnRH is undetectable in jugular blood (176). Clearly, future work will be required to address the functional relevance, if any, of these novel putative GnRH targets.

Conclusion

GnRH is not just a reproductive hormone. Indeed, one of the first functions of GnRH in evolution may have been cardio-active, as shown powerfully in the octopus (9). The diverse

location of GnRH receptors and/or ligand suggests that GnRH may be a major modulator of multiple physiological systems in addition to reproduction. Recent studies suggest that although GnRH may act through a common receptor at the pituitary and these novel sites, the intracellular signaling pathways employed may be different (13,177). Certainly, the presence of a receptor on a particular target does not establish that site as biologically important (for example, olfactory receptors are expressed in the heart (178)). Nevertheless, reconsideration of the potential widespread action that this traditional reproductive hormone may exert, could lead to the generation of novel therapies and encourage due caution when investigating the potential targets of current GnRH therapies (e.g. prostate cancer, endometriosis).

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Figure 1.

(A) Estrogen induced LH surge in the jugular blood and commensurate GnRH surges in the hypophyseal portal system and CSF of a ewe. For details of CSF harvesting, see (179). (B) GnRH receptor-immunoreactive neurons in the murine hippocampus. For details of immunocytochemistry, see (39). (C) Lipopolysaccharide, but not GnRH, induced temperature changes on the skin of the ewe ear. (D) Effect of GHRH and GnRH on GH secretion from an ovariectomized, progesterone treated ewe.

Table 1

Sites outside the hypothalamo-pituitary-reproductive axis that are potential GnRH targets

	Evidence of GnRH receptors	Species	Reference
Nervous Tissue *			
<i>retina</i>	<i>a,b,c</i>	mouse, rat, vole	(88,99,141)
<i>olfactory bulb</i>	<i>a,c</i>	mouse, rat	(37,39,89)
<i>cortex, especially piriform</i>	<i>a,c</i>	mouse, rat	(37,39,140)
<i>lateral septum</i>	<i>a,b,c</i>	mouse, rat	(34,37,39)
<i>preoptic area</i>	<i>b,c,d</i>	mouse, sheep	(31,32,39,82)
<i>arcuate nucleus</i>	<i>b,c,d</i>	mouse, sheep	(25,26,39,82)
<i>hippocampus</i>	<i>a,c</i>	mouse, rat, sheep	(37,39)
<i>amygdala</i>	<i>a,c</i>	mouse, rat, sheep	(37,39)
<i>central gray</i>	<i>a,c</i>	mouse, rat, sheep	(39,129)
<i>cerebellum</i>	<i>b,c,d</i>	mouse, rat	(5,88,95)
<i>spinal cord</i>	<i>d</i>	sheep	(180)
pituitary			
<i>GH</i>	<i>a, d</i>	rat, human	(107,118,122-127,181)
<i>Prolactin</i>	<i>d</i>	rat, human	(118,182,183)
<i>TSH, ACTH</i>	<i>d</i>	rat	(118)
Other			
<i>Kidney</i>	<i>a,b,d</i>	mouse, rat, human	(19,131,139-142,145,175,184,185)
<i>Liver</i>	<i>a,b</i>	mouse, rat, human	(19,89,139-142,145,175,185)
<i>Heart</i>	<i>a,b,c,d</i>	mouse, human	(19,89,139-143,145,159)
<i>Bladder</i>	<i>a,b</i>	mouse, dog, human	(6,139,171,172)
<i>Tooth</i>	<i>b, c</i>	mouse	(186,187)
<i>Adrenal</i>	<i>a,b</i>	mouse, rat, human, cow	(17,139,141,142,145,162-164,185)
<i>Skin</i>	<i>a,b</i>	mouse, rat, dog	(139,142,173)
<i>Skeletal muscle</i>	<i>b</i>	rat, human	(19,139,141,145)
<i>Spleen</i>	<i>a,d</i>	mouse, rat	(89,140,141,145,188,189)
<i>Lymphocytes</i>	<i>a,b,d</i>	mouse, rat, human	(89,174,189,190)

^a binding of a GnRH ligand (e.g. radioactive, biotinylated);^b GnRH receptor mRNA;^c immunoreactive GnRH receptors^d cellular responses (e.g. cell signaling, electrophysiological, secretory)

* There are several other CNS sites that have been identified -see(37,39) for more complete list