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Role of neurotransmitters and neuropeptides in the control of gonadotropin release: A review

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Abstract. Rapid progress has been recorded recently in the understanding of the role of neurotransmitters and neuropeptides in the control of reproduction and on their apparent potential in the regulation of fertility. Peptides, as well as monoamines, are important in the control of lutinizing hormone releasing hormone and gonadotropin release. The input from brainstem noradrenergic neurons as well as dopamine mediated stimulated release of lutinizing hormone. In addition considerable evidence exist for the occurrence of a specific follicle stimulating hormone—releasing factor. A large number of brain peptides affect the secretion of lutinizing hormone releasing hormone and the endogenous opioid peptides appear to have a physiologically important function in restraining the influence on lutinizing hormone releasing hormone release under most circumstances. Vasoactive intestinal peptide and substance *P* stimulate whereas cholecystokinin, neurotensin, gastrin, secretin, somatostatin, α-melanosite stimulating hormone and vasotocin inhibit lutinizing hormone release. Of the inhibitory peptides, cholecystokinin and arg-vasotocin are the most potent. Inhibin injected into the ventricle selectively suppresses follicle stimulating hormone release by a hypothalamic action. Thus the control of gonadotropin release is complex and a number of aminergic and peptidergic transmitters are involved.

Keywords. Neurotransmitters; neuropeptides; LHRH; gonadotropin release.

Introduction

In recent years a large number of peptides, many of which were originally characterized in non-neuronal tissues, have been localized in the central nervous system (CNS). Table 1 lists the major categories of brain peptides described to date. The initial identification of all these peptides in the vertebrate brain were greatly facilitated by the application of radioimmunoassay and immunocytochemistry, which were used to characterize within the CNS peptides previously detected in other vertebrate tissues including those originally assumed to be present only in invertebrates. The possible role of neurotransmitters and brain peptides in the control of anterior pituitary hormone secretion are currently a major research interest in the author's laboratory and some aspects of which will be discussed in this review.

Abbreviations used: CNS, Central nervous system; LHRH, lutinizing hormone releasing hormone; GABA, γ-aminobutyric acid; 5-HT, serotonin; DA, dopamine, PCPA, *p*-chlorophenyl-alanine; VIP, vasoactive intestinal peptide; CCK, cholecystokinin; GnRH, gonadotropin releasing hormone, TRH, thyrotropin-releasing hormone; OVX, ovariecotmised; Met, methionine; Pro, proline; SRIF, somatostatin.

Table 1. Categories of neurotransmitters and peptides present in the mammalian CNS.

Neurotransmitters:	Acetylcholine, norepinephrine, epinephrine, DA, 5-HT. GABA, glutamic acid, aspartic acid.
Hypothalamic Releasing Hormones/Peptides:	TRH GnRH SRIF Corticotropin-releasing hormone Growth hormone releasing hormone Vasopressin, Oxytocin, Enkephalins.
Pituitary peptides:	ACTH, β-endorphin, α-MSH, Prolactin, Growth hormone
Gastrointestinal peptides:	VIP CCK Gastrin, substance *P* Neurotensin, insulin, glucagon Bombesin, secretin, motilin
Others:	Angiotensin II, bradykinin Carnosine, sleep peptide(s), calcitonin, neuropeptide *Y*.

Putative synaptic transmitters controlling gonadotropin release

The lutinizing hormone releasing hormone (LHRH) neurons are in potential synaptic contact with a host of other transmitters, monoaminergic and peptidergic, and consequently a great deal of effort has been directed towards delineating the role of various putative synaptic transmitters in controlling LHRH release. In the median eminence there is an enormous number of terminals which contain various putative transmitters. The median eminence also contains terminals which presumably contain γ-aminobutyric acid, (GABA), histamine, serotonin (5-HT) and norepinephrine (Elde and Hokfelt, 1979).

Dopamine

Early studies with incubation of basal hypothalamus with dopamine (DA) revealed that it released LHRH into the medium (Schneider and McCann, 1969). Recently we were able to show stimulatory effects of DA both in ether-anesthetized and conscious rats following its intraventricular injection. Dopamine or its receptor agonist, apomorphine, was capable of releasing LH in the ovariectomized, steroid primed rat (Vijayan and McCann 1978a; Babu and Vijayan, 1983a). Furthermore, low doses of DA or apomorphine infused intravenously also elicited LH release in steroid-primed and also in ovariectomized animals (Vijayan and McCann, 1978 b). Recent *in vitro* experiments in which LHRH release was measured directly by radioimmunoassay support the stimulatory role for DA in the control of LH release. Incubation of hypothalamic synaptosomes or median eminence tissue with DA resulted in a dose-related increase in LHRH release. These effects were blocked by the dopamine receptor blocker, pimozide (Negro-Vilar *et al.,* 1979).

Norepinephrine

The injection of norepinephrine and epinephrine in the ovariectomized, steroid primed rat released LHRH (Vijayan and McCann, 1978a). Norepinephrine was similarly active

in the median eminence incubation systems and norepinephrine response was blocked by the α-adrenergic receptor blocker, phentolamine (Negro-Vilar *et al.,* 1979).

The role of norepinephrine in stimulation of gonadotropin release was further supported by the extensive turnover studies. The recent study of Rance *et al.* (1981) indicated that the increased turnover of norepinephrine in regions involved in control of LH release at the time of the preovulatory LH surge was preceded by an increased turnover of DA suggesting that both DA and norepinephrine may be involved in the preovulatory release of LHRH.

Serotonin and GABA

The intraventricular injection of 5-HT inhibited LH release indicating a role for this amine in gonadotropin release (Schneider and McCann, 1970; Vijayan *et al.,* 1978a). Stimulation of raphe nuclei to activate 5-HT release in the hypothalamus suppressed pulsatile release of LH (Gallo and Moberg, 1977) indicating that endogenous 5-HT could also suppress LH release. Some doubt was cast on this hypothesis since administration of *p*-chlorophenyl-alanine (PCPA) to block 5-HT biosynthesis failed to modify gonadotropin release in the castrate. It is possible that serotonergic tone may be needed for the expression of the preovulatory discharge of LH, since administration of PCPA or lesions of raphe nuclei to deplete 5-HT stores resulted in an inhibition of estrogen induced LH release (Hery *et al.,* 1976).

The presence of GABA was detected in the hypothalamus as well as the occurrence of GABA receptors in the pituitary. It was therefore not surprising that intraventricular injection of GABA stimulated LH release at relatively high doses (Vijayan and McCann 1978c; McCann *et al.,* 1981). Intraventricular injection of GABA not only altered gonadotropin release but also stimulated the release of norepinephrine and DA from terminals in the median eminence (Negro-Vilar *et al.,* 1980, Babu and Vijayan, 1981). The released catecholamines may be an important function in mediating the effects of GABA on releasing factor discharge.

Peptides

Several peptides present in the hypothalamus have been shown to alter gonadotropin secretion. The opioid peptides, vasoactive intestinal peptide (VIP), neurotensin, substance *P,* cholecystokinin (CCK), gastrin, somatostatin, vasotocin, α-MSH act on the hypothalamus indicating a neurotransmitter/neuromodulatory role for CNS peptides. The strongest candidates among the neuropeptides for neurotransmitter status are substance *P,* closely followed by the enkephalins. Of the hypothalamic hormones, perhaps the best evidence for neurotransmitter function was obtained for thyrotropin-releasing hormone (TRH), followed by gonadotropin releasing hormone (GnRH) but some evidence is available for somatostatin, neurotensin, vasopressin, CCK and others (Emson, 1979; Iversen, 1979; Rehfield, 1980). Such evidence is based on a number of criteria which may be used to attempt to establish neurotransmitter function.

The effect of neuropeptides on pituitary gonadotropin secretion was examined by (i) intraventricular administration of the peptide to animals and the detection of changes in plasma hormone levels, (ii) examining the effect of neuropeptide on the release of hypophysiotrophic hormone from incubated hypothalamic tissue and (iii) examining the effect of a neuropeptide on the release of pituitary hormones from isolated pituitary glands or cells *in vitro.*

The actions of these various peptides and proteins on gonadotropin release in ovariectomized females following their injection into the third ventricle is summarized in table 2. Among these, VIP and substance *P* were stimulatory and most of the others inhibitory in action. VIP elevated LH at nanogram doses (4 to 100 ng) in the castrate animal (Vijayan *et al.,* 1979a) and also released LHRH from the hypothalamic synaptosomes (Samson *et al.,* 1981). Substance *P* required microgram doses to be effective. On the other hand, cholecystokinin inhibited in nanogram doses (Vijayan *et al.,* 1979b) whereas gastrin and neurotensin required higher doses (Vijayan *et al.,* 1978b; Vijayan and McCann, 1979).

Table 2. Effects of intraventricular injection of various peptides on the release of FSH and LH in OVX rats.

Substance	Dosage	FSH	LH
CCK	ng	NE	–
Gastrin	μg	NE	–
VIP	ng	NE	+
Substance P.	μg	NE	+
Neurotensin	μg	NE	–
Opioids	μg	—	–
Angiotensin II	μg	NE	NE
Vasotocin	ng	NE	–
Somatostatin	μg	—	–
α-MSH	μg	NE	–
Secretin	μg	NE	NE
Bombesin	μg	NE	NE
Inhibin	ng	—	NE

+ Increase. – Decrease. NE = No effect.

A number of other peptides have now been evaluated. Vasotocin is extremely potent as intraventricular injection of a minimal effective dose of 40 ng inhibited LH release in ovariectomized rats (Vijayan *et al.,* 1983; Babu Nagesh and Vijayan, E., unpublished results). Arg-vasotocin may play a physiologically significant role if it can be shown to be present in the mammalian brain. Recent studies suggest that it is not present in mammalian pineals including the brattleboro rat (Negro-Vilar, *et al.,* 1982). None of these peptides altered gonadotropin release by hemipituitaries or dispersed pituitary cells *in vitro.*

Bombesin, a tetradecapeptide, did not have impressive effects on gonadotropin secretion, however, intraventricular bombesin produced significant depletion of prolactin. On the other hand, secretin another peptide found in the hypothalamus suppressed LH release in ovariectomized rats (Babu and Vijayan, 1983b). Release

of LH by hemipituitaries was unaffected by either peptides *in vitro.* Therefore, it cannot be stated that bombesin or secretin have any clear role in gonadotropin secretion.

Angiotensin II, another peptide probably found in the median eminence, released LH from hemipituitaries of ovariectomized rats at doses of 0·2 to 2 μg/ml. Intraventricular injection of angiotensin II was ineffective in modifying LH in the ovariectomised (OVX) animal. However, in OVX-estrogen primed animals it dramatically elevated LH levels (Steele *et al.,* 1981). Angiotensin II receptors were demonstrated in the anterior pituitary suggesting that the action of the peptide in releasing LH from pituitaries incubated *in vitro* may have physiological significance (Mukherjee *et al.,* 1982). Angiotensin II was implicated as to have a physiological function in the release of LH.

The discovery of opioid peptides in the brain provided a new approach towards understanding of neural mechanisms controlling reproduction. Episodic LH release was rapidly suppressed when *ß*-endorphin was injected into the third ventricle of castrated male rats (Kinoshita *et al.,* 1980) or OVX rats (Vijayan, E., unpublished results). The met-enkephalin analog, (D-Met^2, Pro^5) enkephalinamide, blocked ovulation and the preovulatory LH surge when injected intracerebrally into the lateral ventricle of proestrous rats (Koves *et al.,* 1981). The opiate receptor antagonists not only suppressed competitively the effects of opiates and endogenous opioid peptides, but invariably evoked LH release on their own when tested in several species including man (Kalra, 1982). The ease with which Naloxone, an opiate receptor antagonist, stimulated LH release provided the most direct evidence of involvement of endogenous opioid peptide in the control of gonadotropin secretion. It is generally believed that endogenous opioid peptides normally exert an inhibitory influence on LH release under a variety of circumstances and may play an important role in gonadotropin secretion (Kalra, 1982).

Inhibin, the peptidic factor apparently secreted by ovary and testis to inhibit FSH release was demonstrated to have a selective effect in suppressing FSH release at the hypothalamic site. Microinjection of purified inhibin preparations into the third ventricle in castrate animals resulted in a suppression of FSH but not LH release (Babu *et al.,* 1981; Lumpkin *et al.,* 198 1a). The action was probably exerted on the CNS since test doses of LHRH was equally active in releasing FSH and LH in control and in inhibin treated animals. Since the inhibin injected into the ventricle preferentially inhibited FSH release the results were explained in terms of inhibition of the release of an FSH releasing factor. In a more recent study, human seminal plasma inhibin suppressed both FSH and LH in immature rats of either sex (Babu and Vijayan, 1983c).

Somatostatin (SRIF) terminals are intermingled amongst the terminals of the LHRH neurons in the median eminence. Intraventricular injection of somatostatin produced an inhibition of LH and FSH release by blocking LHRH release in the median eminence region (Lumpkin *et al.,* 1981b). It is quite likely that the various releasing factor neurons interact to augment or inhibit each others activity in the hypothalamus (Piva *et al.,* 1979).

α-MSH, a fragment of the propiomelanocortin sequence, has recently been found to suppress LH release in OVX rats after its intraventricular injection. The inhibitory effects of α-MSH are most likely exerted in the brain, presumably on structures adjacent to the third ventricle (Khorram *et al.,* 1984).

Conclusions and future trends

The data reviewed here indicate that intraventricular injections of a variety of transmitters/peptides other than the releasing hormones have powerful actions on pituitary gonadotropin release probably by altering the discharge of hypothalamic releasing and/or inhibiting neurons. Since the peptides are localized to the hypothalamus, a site near the ventricle, an action on hypothalamic neuronal activity is likely. However, since most of the peptides are localized also to other sites in the brain, an action at these more distant sites cannot be ruled out. At the present time the true identity, diversity and activity of neuropeptides in both neural and neuroendocrine systems is still far from clear. From what has already emerged it is certain that further interesting revelations are just around the corner awaiting future research. The generation of neuropeptides from larger precursor molecules, their further processing prior to release, and their degradation by peptidases after release will require careful analysis and make some questions particularly difficult to answer without the development of new or improved methodology. The interaction of the peptides with other transmitter system in the brain is an area which requires a great deal of further study. Delineation of such interactions should aid in the understanding of the intricacy of CNS function. We know that some peptides are hormones, some are very likely to be neuromodulators or neurotransmitters and several appear to have separate neural and endocrine functions in different tissues. But what are the mechanisms of action in the brain? Are they short-term neuromodulators or modulators of cellular metabolism? Perhaps research undertaken in the near future will provide the answers, and more than likely, reveal the role of these peptides both in health and reproduction and offer therapeutic approaches in a variety of disease states.

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