
Responses in the hypothalamic monoaminergic system activity in ewes to β -endorphin, CRF and their antagonists

Franciszek Przekop and Dorota Tomaszewska

The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 3 Instytucka St., 05-110 Jabłonna n. Warsaw, Poland

Abstract. This article reviews data concerning the action of opioidergic and monoaminergic system on LHRH secretion. Generally, in anestrus ewes β -endorphin and/or corticoliberin significantly change extracellular concentrations of monoamine metabolites in the MBH-ME, but in estrus ewes both β -endorphin and CRF alters also dopamine, noradrenaline and serotonin levels. Responses of catecholaminergic and serotonergic system in the MBH-ME to naloxone or CRF-antagonist depend, to a large degree, on the phase of reproduction. In anestrus ewes subjected to stressful stimuli an opiate receptor blocker, naloxone, and CRF-antagonist attenuate the stress-induced activity of catecholaminergic and serotonergic system in the MBH-ME; in non-stressed animals they suppress only serotonergic system activity in this structure. No clear explanation can be offered now for either differences in response of catecholaminergic and serotonergic system in the MBH to β -endorphin and CRF in various periods of reproduction or for differences in the responses of these systems to CRF antagonist and naloxone in non-stressed and stressed ewes. It has been suggested that the responses in monoaminergic system activity are highly dependent upon the physiological state of the animal and that β -endorphin and corticoliberin may indirectly modulate LHRH and other hypothalamic hormone secretion by monoaminergic systems.



Mini-review

Key words: DA, NE, 5-HT, metabolites of monoamines, β -endorphin, CRF, stress, hypothalamus-median eminence

INTRODUCTION

The regulation of hypothalamic LHRH and other neurohormone secretion is associated with a complex interplay among various excitatory and inhibitory neurotransmitters within the hypothalamus. Regarding LHRH release, evidence has been accumulated over recent years in support of a functional relationship between opioidergic (Domański et al. 1991, Rasmussen 1991, Fink 1994), corticoliberinergic (Rivest and Rivier 1995) and monoaminergic systems of the hypothalamus (Kalra and Kalra 1983) in regulation of both the basal LH secretion and the preovulatory LHRH/LH surge. It has been demonstrated that among several neurotransmitters whose axon terminals synapse directly on LHRH neurones, are those originating from dopaminergic (Leranth et al. 1988, Chen et al. 1989 a, b, Kuljis and Advis 1989, Horvarth et al. 1993), serotonergic (Kiss and Halasz 1985), β -endorphinergic (Chen et al. 1984, Thind and Goldsmith 1988) and corticoliberinergic neurones (MacLusky et al. 1988), thus suggesting that all of the above-mentioned systems may directly affect LHRH release. Interconnection between opioidergic and dopaminergic neurones in the hypothalamus (Fitzsimmons et al. 1992, Horvath et al. 1992) provides potential opportunities that opioidergic neurones in this structure might act on LHRH cells through interpolated DA cells. Numerous neuropharmacological studies indicate also that opioid peptides may affect hypothalamic LHRH neurones through catecholaminergic and serotonergic systems. It has been shown that stimulation of opioid receptors prior to a critical period of proestrus in rats with β -endorphin (Leadem and Kalra 1985) or opioid agonists (Köves et al. 1981), blocks the preovulatory LHRH/LH surge with a concomitant decrease in hypothalamic DA release (Lohse and Wuttke 1981, Heijna et al. 1991). On the other hand, naloxone-induced LH release in this species can be blocked by an α -adrenergic receptor antagonist or by suppression of hypothalamic catecholamine levels (Kalra and Simpkins 1981, Kalra and Karla 1983). *In vitro* studies have demonstrated that the mode of action of dopamine on LHRH release

from the nucleus infundibularis-median eminence of rats depends upon opioid activity. It has been found that dopamine inhibition of LHRH release from this structure is mediated by increased endogenous opioidergic tone and that blocking this enhanced endorphinergic activity allows a stimulatory dopaminergic effect on LHRH release to be expressed (Rasmussen 1991). In another series of studies on rats, it has been demonstrated that opioid peptides may stimulate rather than inhibit release of LH, probably acting indirectly through noradrenergic and/or serotonergic systems located on LHRH neurones (He and Barraclough 1991, 1993). In ewes, similarly as in most experiments done in rats, β -endorphin (Curlevis et al. 1991, Conover et al. 1993) and morphine (Currie et al. 1991) suppress LH release, while opioid antagonists stimulate this hormone secretion (Malven et al. 1990, Whisnant et al. 1991). The obtained results show that the nucleus infundibularis-median eminence is a major control site where opioid peptides exert their inhibitory influence on LHRH release (Domański et al. 1991, Conover et al. 1993). Transient antagonism of endogenous opioid peptides with systemic naloxone administration (Goodman et al. 1995a) or implants of opioid antagonists into the mediobasal hypothalamus or preoptic area disinhibits release of LHRH (Goodman et al. 1995) and LH (Malven et al. 1990, Weesner and Malven 1990, Whisnant et al. 1991) in ewes. These studies revealed that opioid peptides might also affect the LHRH system at the level of LHRH perykaria located in the preoptic area. Immunoneutralization of endogenous β -endorphin and met-enkephalin relieves the inhibition of LH release in the luteal phase of the estrous cycle in ewes thus further supporting the inhibitory influence of opioid peptides on LHRH/LH secretion (Weesner and Malven 1990). However, the mode of action of opioid peptides on LHRH release in this species is not fully clarified. Although it is generally believed that they act directly on LHRH cell bodies and neuronal terminals, it cannot be excluded that the inhibitory tone of these peptides on LHRH release may be transmitted indirectly at least in part, through the noradrenergic system in the nucleus in-

fundibularis-median eminence. It has been established that on the day of estrous in ewes, decreased release of β -endorphin allows the expression of NE activity in the nucleus infundibularis-median eminence and the augmentation of NE tone triggers the preovulatory LHRH surge (Domański et al. 1991).

Further studies in our laboratory tested the hypothesis that dopaminergic and β -endorphinergic activity in the nucleus infundibularis-median eminence might also be a component of the neuronal circuit modulating preovulatory LHRH surge. The preliminary results suggest that the onset of LHRH/LH surge in ewes may be related to the withdrawal or reduction of the inhibitory tone of DA and β -endorphin on LHRH neurones in the nucleus infundibularis-median eminence (Chomicka et al. 1994).

Numerous neuropharmacological studies over last years have been made towards a better understanding of the catecholaminergic mechanism that governs seasonal reproduction. After decades of controversy on the function of the central catecholaminergic system in the control of LHRH/LH release, a consensus now emerges from most neuropharmacological works in favour of a predominantly inhibitory action of catecholamines during the anestrus period (Meyer and Goodman 1985, Meyer and Goodman 1986, Havern et al. 1994). On the basis of direct measurements of catecholamines and LHRH release, these results have been reevaluated. First of all, it has been established quite recently that estradiol induced a decrease in NE levels in the preoptic area with concomitant suppression of LH release in anestrus ewes (Goodman et al. 1995b), this statement is not consistent with the hypothesis that estradiol decreases the LH pulse frequency in anestrus ewes by augmenting an inhibitory noradrenergic tone in this structure. Another important studies on the neuroendocrine events that underlie generation of seasonal reproductive cycles revealed that dopaminergic activity in the nucleus infundibularis-median eminence changes in a characteristic manner during the anestrus cycle (Chomicka et al. 1994). In early anestrus, the extracellular concentration of DA is low,

then it progressively increases. The termination of seasonal anestrus is preceded by a marked rise in LHRH release with concomitant attenuation of DA efflux. These data suggest that role of dopaminergic system in the control of LHRH/LH release may change in different period of non-breeding season.

Similarly, the serotonin-induced changes of LH release are largely dependent upon the physiological state of sheep (Dailey et al. 1987). The precise nature of the monoaminergic system in the hypothalamus in the control of LHRH secretion remains to be defined.

Most recent studies also suggest that CRF may act on LHRH cells, at least in part, through the monoaminergic system in the hypothalamus. The basis for this concept is that intraventricular or systemic injection of CRF suppresses hypothalamic LHRH secretion (Rivest and Rivest 1995), facilitates noradrenaline and dopamine release (Emoto et al. 1993, Levicky and Dunn 1993) and changes the metabolic activity of catecholamines (Dunn and Berridge 1990). It must be mentioned here that the role of CRF in mediating the activity of the hypothalamic-pituitary-ovarian axis may vary among species and may be species-specific. Indeed, in the monkey, systemic administration of CRF rapidly decreases LH levels. These results are quite different from those obtained in rodents in which systemic CRF administration does not significantly alter gonadotropin secretion (Rivest and Rivest 1995). In sheep, CRF displays a rather stimulatory effect on LH release. In ovariectomized, estradiol-treated ewes, CRF causes a dose-related increase in LH secretion (Naylor et al. 1990). The question whether a CRF-antagonist alters catecholaminergic and serotonergic system activity in the hypothalamus and LHRH/LH release is still without answer.

The studies outlined in this review were designed to analyse: (1) the effect of β -endorphin and CRF on the catecholaminergic and serotonergic system activity in the MBH-ME of anestrus and estrus ewes, and (2) the influence of opioid receptor blocker, naloxone, and CRF-antagonist on the monoaminergic system activity in the MBH-ME of anestrus stressed and unstressed ewes.

THE EFFECT OF β -ENDORPHIN ON THE EXTRACELLULAR CONCENTRATION OF MONOAMINES AND THEIR METABOLITES IN THE MBH-ME OF ANESTROUS AND ESTROUS EWES

In anestrus ewes during the early non-breeding season, β -endorphin administered locally into the MBH-ME does not evidently change catecholamine and serotonin release (Table I). The lack of changes in the concentration of MHPG also indicates that β -endorphin does not exert an important role on the metabolic activity of the noradrenergic system in the MBH-ME of anestrus ewes. On the other hand, a marked increase of extracellular DOPAC, HVA and 5-HIAA suggests enhancement of metabolic activities in the dopaminergic and serotonergic systems in the MBH-ME of these animals. These results lead to the conclusion that the specific action of β -endorphin in MBH-ME of anestrus ewes is associated mainly with stimulation of metabolic activities in the dopaminergic and serotonergic systems in the MBH-ME rather than

in the release of neurotransmitters. The physiological significance of this phenomenon on the neuroendocrine processes in the hypothalamus remains to be resolved.

In ewes prior to a preovulatory LH surge, β -endorphin substantially increases the extracellular concentration of DA in the MBH-ME of most animals. The marked rises in the level of this amine may result from an increase of exocytotic release of DA or attenuation of extraneuronal dopamine uptake. In the rat MBH, β -endorphin and other opiates suppress dopamine release (Lohse and Wuttke 1981, Reymond et al. 1983, Heijna et al. 1991), however, in the ventral tegmental area, opioid agonist caused dose-dependent increases in DA release and extracellular dopamine metabolite concentrations (Devine et al. 1993), thus indicating that dopaminergic system in different structures react in a specific manner to exogenous opiates. Data from *in vitro* studies have also shown that exogenous opioid can either stimulate or inhibit DA and NE release from the median eminence (Rasmussen et al. 1988). All of the mentioned results suggest that opioid peptides action in the MBH-ME on DA release is dependent on the physiological activity of the dopaminergic system and may be also species-spe-

TABLE I

Changes in monoamines and their metabolites concentration in the MBH-ME perfusates in CRF and β -endorphin treated ewes during different reproduction stages. Values are means \pm SD; *significant differences ($P \leq 0.05$, Wilcoxon test $n = 5$)

Group	% change						
	NE	DA	5-HT	MHPG	DOPAC	HVA	5-HIAA
Estrus prior to the preovulatory LH surge							
CRF	447 \pm 83*	-	109 \pm 48*	81 \pm 45*	83 \pm 48*	84 \pm 39*	35 \pm 14
β -endorphin	32 \pm 17	268 \pm 59*	55 \pm 31	-69 \pm 14*	-69 \pm 15*	-70 \pm 15*	-71 \pm 15*
Estrus during preovulatory LH surge							
CRF	21 \pm 16	-	36 \pm 16	52 \pm 15*	-10 \pm 7	31 \pm 18	45 \pm 11*
β -endorphin	-62 \pm 11*	161 \pm 40*	31 \pm 19	-70 \pm 16*	-71 \pm 17*	-73 \pm 17*	-74 \pm 17*
Anestrus							
CRF	-	-	21 \pm 9	136 \pm 64*	106 \pm 46*	191 \pm 40*	94 \pm 31*
β -endorphin	-	-	39 \pm 18	14 \pm 8	83 \pm 35*	79 \pm 36*	80 \pm 36*

cific. Taken together, the results from anestrous and estrous ewes also indicate that different mechanisms might control DA release in various periods of reproduction. In the estrous ewes prior to a preovulatory LH surge β -endorphin does not essentially change the extracellular NE concentration. This suggests that the release of noradrenaline from nerve terminals is independent of opioid action. However, the failure of exogenous β -endorphin to change NE output does not exclude the possibility that β -endorphin may affect a release of noradrenaline. Such a suggestion has been implied from previous studies, which indicate that high activity of the β -endorphinergic system in the nucleus infundibularis-median eminence of ewes prior to the preovulatory LH surge is responsible for suppression of NE and LHRH release (Domański et al. 1991). In concordance with this observation, high activity of the opioidergic system in the MBH-ME may be one of the important factor(s) which masks the inhibitory effect of exogenous β -endorphin on NE release. The opposite patterns of DA and NE response to β -endorphin in estrous ewes prior to a preovulatory LH surge suggest that opioids play different roles in DA and NE release.

The lack of significant changes of 5-HT during β -endorphin administration shows that this drug may play only a marginal role in 5-HT release in estrous ewes.

A significant decrease in the extracellular concentrations of catecholamine and serotonin metabolites in the MBH-ME of estrous ewes during β -endorphin administration indicates that β -endorphin decreases the metabolic activity in the monoaminergic system in MBH-ME. Works done on rats demonstrate that the extracellular concentrations of catecholamine and serotonin metabolites do not directly reflect dopamine, noradrenaline and serotonin release. Indeed, only a part of the extracellular metabolites of catecholamines in the rat brain have their origins in released amines (Zetterström et al. 1988). In this species, a large part of extracellular dopamine metabolite formation takes place in the intraneural pool of newly synthesized DA. In the brain region innervated by noradrenergic neurones,

extracellular DOPAC can reflect oxidative deamination of axoplasmic dopamine in noradrenergic terminals. It also seems that extracellular MHPG are not closely linked with noradrenaline release. Similarly, a recent study suggests that the rise of 5-HT in the rat brain is not directly correlated with serotonin metabolism. Such conclusion is in agreement with results done on the rat (Devaud et al. 1992).

A stimulatory effect of β -endorphin on DA release in the MBH-ME also occurs during the preovulatory LH surge.

However, in these animals β -endorphin clearly decreases extraneuronal NE concentration; this suggest that opioid peptides can suppress NE release from the nerve terminals in the MBH-ME during the preovulatory LH surge. The differences in the response of NE released in various states of the estrous cycle to β -endorphin administration is probably associated with changes of activity of various excitatory and inhibitory neurotransmitters and neurohormones within the hypothalamus. It has been established that opioid receptor-mediated NE release from the hypothalamus in the rat appears not to be a general phenomenon. *In vitro* studies show that NE release from the rat medial hypothalamus is not modulated by any of the opioid receptors (Heijna et al. 1991) while others indicate that naloxone, depending on the dose used, can stimulate or inhibit NE release from the median eminence (Rasmussen et al. 1991). It has been stated that chronically administered morphine decreased NE levels in the mediobasal hypothalamus in male rats, whereas it did not alter the NE turnover in the preoptic area-anterior hypothalamus (Gabriel et al. 1988). Interestingly, in these ewes, similarly as in estrous animals prior to preovulatory LH surge, β -endorphin did not remarkably affect 5-HT release. The decrease in the concentration of extracellular MHPG, DOPAC, 5-HIAA suggests that β -endorphin profoundly suppresses a metabolic process in the MBH-ME catecholaminergic and serotonergic systems.

In conclusion, these results indicate that β -endorphin acts in a specific manner on dopaminergic, noradrenergic and serotonergic system activity in

the MBH-ME of ewes during different stages of reproduction. It is reasonable to suggest that β -endorphin can affect hypophysiotropic hormone release including LHRH indirectly through catecholaminergic system in the MBH-ME.

THE EFFECT OF CRF ON EXTRACELLULAR CONCENTRATIONS OF NE, 5-HT AND MONOAMINE METABOLITES IN THE MBH-ME OF ANESTROUS AND ESTROUS EWES

CRF caused a significant elevation in the extracellular concentrations of catecholamine and serotonin metabolites in anestrous ewes, but had no evident effect on noradrenaline and serotonin levels (Tomaszewska et al. 1992). These data indicate that CRF stimulates metabolic activity in the MBH-ME catecholaminergic and serotonergic systems, but does not affect the release of noradrenaline and serotonin in anestrous ewes (Table I). In estrous ewes, prior to the preovulatory LH surge, CRF induces an increase of the extracellular concentration of NE and 5-HT. It is reasonable to suggest that the rises of extracellular NE and 5-HT levels may result from an increase in the exocytotic release of these amines, or attenuation of extraneuronal uptake of these neurotransmitters. The increase of MHPG and DOPAC levels indicates that CRF facilitates metabolic activity in the catecholaminergic system in the MBH-ME. The question whether the rise in extracellular concentration of DOPAC and HVA is followed by an increase in the release of DA awaits to be resolved.

In ewes during the preovulatory LH surge, when NE release is elevated, the specific action of CRF on the noradrenergic system in the MBH-ME is linked with stimulation of metabolic activity of NE rather than with changes in the release of this amine. On the other hand lack of changes in extracellular DOPAC and HVA concentration suggest that CRF does not substantially affect metabolic activity in

dopaminergic system in the MBH-ME of estrous ewes during preovulatory LH surge. In these animals, CRF did not change 5-HT level, but markedly increased 5-HIAA concentration. At present, we can not explain the dissociation in extracellular concentration between 5-HT and 5-HIAA in this animals. It is generally accepted that an important measure of change in neural function is obtained from determining treatment effects on both metabolite levels as well as on turnover. However, recent studies indicate that the increase of 5-HT in the rat brain is not directly correlated with 5-HT metabolism (Devaud et al. 1992).

These studies indicate that responsiveness of monoaminergic systems in the MBH-ME to CRF is different during various phases of estrous and in anestrous ewes. Generally, CRF exerts a stimulatory effect on NE and 5-HT release in estrous ewes prior to the preovulatory LH surge and increases metabolic activity in noradrenergic and serotonergic nerve terminals during different phases of the estrous cycle of ewes and in anestrous animals. Except for DOPAC and HVA in some estrous ewes during preovulatory LH surge, CRF substantially increases extracellular DOPAC and HVA thus suggesting stimulatory influence on metabolic activity in the MBH-ME dopaminergic system.

There are relatively few studies concerning the effect of CRF on the monoaminergic system activity in the hypothalamus in other species. In rats the administration of CRF increases catecholamines and their metabolites in different parts of the brain, including the hypothalamus (Dunn and Berridge 1990). However, measurements in homogenates of the tissue do not distinguish between the intra- and extracellular sources of neurotransmitters and their metabolites. Using the microdialysis technique it has been shown that CRF produces dose-dependent increases in dialysate concentrations of DA and NE (Emoto et al. 1993, Levicky and Dunn 1993).

In conclusion, present results indicate that CRF administered locally into the MBH-ME in ewes facilitates NE and 5-HT release in this structure in estrous ewes prior to a preovulatory LH surge. CRF also stimulates metabolic activities in catechola-

minergic system in the MBH-ME in anestrous and estrous ewes, except in the dopaminergic system in ewes during the preovulatory LH surge. It is suggested that responsiveness of monoaminergic systems in the MBH-ME to CRF is dependent, to a large degree, on the physiological state of animals and that CRF may through monoaminergic systems in the MBH-ME, modulate hypothalamic hormone secretion, including LHRH.

THE INFLUENCE OF NALOXONE AND CRF-ANTAGONIST ON MONOAMINERGIC SYSTEM ACTIVITY IN THE MBH-ME OF ANESTROUS EWES UNDER NORMAL AND STRESSFUL CONDITIONS

In sheep subjected to footshocks, 5-HT neurones responded with massive, sustained release of serotonin into the MBH-ME, but there was no effect on DA and NE efflux (Tomaszewska et al. 1995). However this stress increased metabolic activity in the catecholaminergic and serotonergic systems in the MBH-ME (Table II). Now it is well established from experiments on rats that stressful stimuli activate catecholaminergic and serotonergic system activity (Adell et al. 1988, Imperato et al. 1992, Vetrugno et al. 1993, Pacak et al. 1995, Pol et al. 1995). The mode of response of the monoamine

release in stressed rats is dependent to a large degree upon the physiological state, nature of the stress and may differ in discrete regions of the brain. The regional and species-specific response of monoaminergic systems to stressful stimuli is probably associated with multifactorial interplay among excitatory and inhibitory neurotransmitters within the hypothalamus. In this aspect, the present experiments represent a study that analyses the response of catecholaminergic and serotonergic systems in non-stressed and stressed ewes treated locally in the MBH-ME with opiate receptor antagonist, naloxone, and CRF-antagonist. The decrease of 5-HT and 5-HIAA during naloxone administration in stressed and non-stressed ewes shows that blocking opioid receptors leads to suppression of serotonergic system activity in normal situation as well as under stressful conditions.

Taken together, these findings suggest that the release and metabolism of 5-HT in non-stressed and stressed ewes is modulated *via* opioid receptors located on serotonergic nerve terminals in the MBH-ME. There are relatively few studies on the effect of opioid blocking agents on serotonergic activity in other species. In orchidectomized rats, the 5-HT concentration in the hypothalamus is significantly elevated by both naloxone and morphine treatment; however these compounds have no significant effect on the turnover of this amine (Gopalan et al. 1991). In female rats, nalmefrone blocks the increase in 5-HT turnover in the medial preoptic area

TABLE II

Changes in monoamines and their metabolites concentration in MBH-ME perfusates in stressed and non-stressed ewes during naloxone or CRF-antagonist administration. Values are means \pm SD; a significant differences vs. control ewes b significant differences vs. stressed ewes ($P \leq 0.05$, Wilcoxon test $n = 6$)

Group	% change				
	5-HT	MHPG	DOPAC	HVA	5-HIAA
Stress	115 \pm 43 a	134 \pm 68 a	341 \pm 78 a	1305 \pm 150 a	587 \pm 78 a
Naloxone	- 56 \pm 16 a	- 5 \pm 4	40 \pm 15	- 46 \pm 20	- 58 \pm 13 a
Stress + naloxone	- 53 \pm 23 a,b	- 97 \pm 12 a,b	- 48 \pm 18 a,b	- 86 \pm 21 a,b	- 85 \pm 25 a,b
CRF-antagonist	- 96 \pm 12 a	82 \pm 28 a	- 10 \pm 6	- 22 \pm 8	- 47 \pm 12 a
Stress + CRF-antag.	85 \pm 15 a,b	104 \pm 44 a,b	18 \pm 31 b	40 \pm 14 a,b	20 \pm 9 b

and in the ventromedial nucleus induced by estrogen (Johnson and Crowley 1984).

The effect of naloxone on the catecholaminergic system in the MBH-ME of ewes is less clear. This is because the catecholamines in the perfusates could not be measured reliably during vehicle or naloxone perfusion, and that changes in concentration of extracellular metabolites are not statistically significant during naloxone administration. Also, experiments performed on rats give no unequivocal results. For instance, it was reported that opioids suppress DA release (Kalra and Kalra 1983), *in vitro* studies indicate that naloxone or morphine can either stimulate or inhibit DA and NE release from the median eminence, depending on the dose of the drugs used (Rasmussen et al. 1988). In contrast to non-stressed ewes, naloxone administered during stressful stimuli profoundly decreases the concentration of catecholamine metabolites. At present we can not offer a clear explanation for differences in the response of catecholamine metabolites in non-stressed and stressed animals. It seems, therefore, possible that stress-induced changes of opioids and POMC-derived peptide release may modify other peptidergic and aminergic system(s) which affect the metabolism of catecholamines. Such a suggestion was presented in our previous work to explain the stimulatory effect of β -endorphin in non-stressed ewes and suppression action of this peptide on cortisol secretion in stressed animals (Przekop et al. 1990).

Although CRF receptors have been identified within the hypothalamus (Webster et al. 1991), their role in the mediating effect on neurotransmitter release remains obscure. In non-stressed ewes CRF-antagonist, similar as naloxone decreases extracellular 5-HT and 5-HIAA concentration but has no significant effect on catecholamine metabolites level. In stressed animals it attenuates stress-induced release of 5-HT and metabolic activity in catecholaminergic and serotonergic system in the MBH-ME. These results suggest that mentioned above changes in monoaminergic system activity in the MBH-ME of non-stressed and stressed ewes during CRF-antagonist perfusion is mediated, at

least in part, through the blockade of CRF-receptors on the serotonergic and catecholaminergic nerve terminals or interpolated neurones which act on monoaminergic systems. The differences in the responses of catecholaminergic and serotonergic system in the MBH-ME to CRF-antagonist await to be resolved. In rats, intraventricular administration of CRF-antagonist has no evident effect on basal release of noradrenaline from the prefrontal cortex but attenuates the immobilisation-induced release of this amine (Shimuzu et al. 1994).

In conclusion the presented results indicate that stress-induced 5-HT release and metabolic activity in the MBH-ME of anestrous ewes is attenuated by CRF-antagonist. In non-stressed animals CRF-antagonist decreases only serotonergic system activity in this structure.

CONCLUSIONS

Evidence has been accumulated over last years in support of a functional relationship between the opioidergic, corticoliberinergic and monoaminergic systems in the control of hypothalamic neurohormone release, including LHRH. In current studies on anestrous and estrous ewes an initial step has been established that β -endorphin and corticoliberin act in specific manner on the catecholaminergic and serotonergic system activities in the MBH-ME, as manifested by extracellular concentration of monoamines and their main metabolites. An opiate receptor blocker, naloxone, and CRF-antagonist attenuate the stress-induced activity of the catecholaminergic and serotonergic systems in the MBH-ME of anestrous ewes but in non-stressed animals they suppress only serotonergic activity in this area. The most likely common interpretation of these results is that β -endorphin and CRF can act through specific receptors located on catecholaminergic and serotonergic systems in the MBH-ME to modulate the activity of these systems. No clear explanation can be offered now for observed differences in the response of catecholaminergic and serotonergic system in the MBH-ME of ewes to β -endorphin and CRF in various periods of repro-

duction. The similar statement seems to be valid regarding the differences in the responses of these systems to naloxone and CRF-antagonist in non-stressed and stressed ewes. It is suggested that responses of monoaminergic systems to β -endorphin and CRF in large degree are dependent upon the physiological state of animals and that opioid peptides and CRF may modulate hypothalamic hormone secretion, including LHRH, by monoaminergic system in the MBH-ME.

ACKNOWLEDGEMENT

This work was supported by KBN Grant 5PO6K 04508.

REFERENCES

- Adell A., Garcia-Marquez C., Amario A., Gelpi E. (1988) Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their responses to a further acute stress. *J. Neurochem.* 50: 1678-1681.
- Chen W.P., Witkin J.W., Silverman A.J. (1989a) Gonadotropin releasing hormone (GnRH) neurons are directly innervated by catecholamine terminals. *Synapse* 3: 289-290.
- Chen W.P., Witkin J.W., Silverman A.J. (1989b) β -Endorphin and gonadotrophin-releasing hormone synaptic input to gonadotrophin-releasing neurosecretory cells in the male rat. *J. Comp. Neurol.* 286: 85-95.
- Chomicka L.K., Gajewska A., Przekop F. (1994) Release of luteinizing hormone-releasing hormone (LHRH) and dopamine (DA) by the nucleus infundibularis/median eminence (NI/ME) during seasonal anestrus in ewes. 3-rd International Congress of Neuroendocrinology, Budapest, Abstracts, p. 66.
- Chomicka L.K., Wolińska-Witort E., Przekop F. (1994) Release of LHRH, β -endorphin and dopamine by the nucleus infundibularis/median eminence during the oestrous cycle in the ewe. *J. Endocrinol.* 130 (Suppl.)2: 2.
- Conover C.D., Kuljis R.O., Rabii J., Advis J.P. (1993) β -endorphin regulation of luteinizing hormone releasing hormone at the median eminence in ewes: immunocytochemical and physiological evidence. *Neuroendocrinology* 57: 1182-1195.
- Curlevis J.D., Naylor A.M., Rhind S.M., McNeilly A.S. (1991) Effects of β -endorphin on pulsatile luteinizing hormone and prolactin secretion during follicular phase in the ewe. *J. Neuroendocrinol.* 3: 123-126.
- Currie W.D., Joseph I.B.J.K., Rawlings N.C. (1991/92) Morphine, naloxone and gonadotropin surge in the ewe. *J. Reprod. Fertil.* 2: 407-414.
- Dailey R.A., Deaver D.R., Goodman R.L. (1987) Neurotransmitter regulation of luteinizing hormone and prolactin secretion. *J. Reprod. Fertil. (Suppl.)*34: 17-26.
- Devaud L.L., Hollingsworth E.B., Cooper B.R. (1992) Alterations in extracellular and tissue levels of biogenic amines in rat brain induced by serotonin₂ receptor antagonist, ritanserin. *J. Neurochemistry* 59: 1459-1466.
- Devine D.P., Leone P., Pocock D., Wise R.A. (1993) Differential involvement of ventral tegmental Mu, Delta and Kappa opioid receptor in modulation of basal mesolimbic dopamine release: in vivo microdialysis studies. *J. Pharmacol. Exp. Ther.*266: 1236-1246.
- Domański E., Chomicka L.K., Ostrowska A., Mateusiak K. (1991) Release of luteinizing hormone-releasing hormone, β -endorphin and noradrenaline by the nucleus infundibularis-median eminence during periovulatory period in the sheep. *Neuroendocrinology* 54: 151-158.
- Dunn A.J., Berridge C.W. (1990) Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Rev.* 15: 71-100.
- Emoto H., Yokoo H., Yoshida M., Tanaka M. (1993) Corticotropin-releasing factor enhances noradrenaline release in the rat hypothalamus assessed by intracerebral microdialysis. *Brain Res.* 601: 286-288.
- Fink G. (1994) Molecular principles from neuroendocrine models: steroid control of central neurotransmission. *Prog. Brain Res.* 100: 139-147.
- Fitzsimmons M.D., Olschowska J.A., Wiegand S.J., Hoffman G.E. (1992) Interaction of opioid peptide-containing terminals with dopaminergic perikarya in the rat hypothalamus. *Brain Res.* 581: 10-18.
- Gabriel S.M., Clark J.T., Kalra P.S., Kalra S.P., Simpkins J.W. (1988) Chronic morphin and testosterone treatment: effects on norepinephrine and serotonin metabolism and gonadotropin secretion in male rats. *Brain Res.* 447: 200-203.
- Goodman R.L., Parfitt D.B., Evans N.P., Dahl G.E., Karsch F.J. (1995a) Endogenous opioid peptides control the amplitude and shape of gonadotropin-releasing hormone pulses in the ewe. *Endocrinology* 136: 2412-2420.
- Goodman R.L., Robinson J.E., Kendrick K.M., Dyer R.G. (1995b) In the inhibitory action of estradiol on luteinizing hormone pulse frequency in anestrus ewes mediated by noradrenergic neurons in the preoptic area? *Neuroendocrinology* 61: 284-292.
- Gopalan C., Currie I.S., Gilmore D.P. (1991) Effects of opiates on hypothalamic amine turnover and LH levels in castrated rats. *Biogenic Amines* 8: 141-148.
- Havern R.L., Whisnant C.S., Goodman R.L. (1994) Dopaminergic structures in the ovine hypothalamus mediating estradiol negative feedback in anestrus ewes. *Endocrinology* 134: 1905-1914.
- He J.R., Barraclough C.A. (1991) Morphine but not naloxone enhances luteinizing hormone releasing hormone neuro-

- nal responsiveness to norepinephrine. *J. Neuroendocrinol.* 4: 91-99.
- He J.R., Barraclough C.A. (1993) Morphine amplifies norepinephrine (NE)-induced release but blocks NE increases in LHRH mRNA levels: comparison of responses obtained in ovariectomized, estrogen-treated normal and androgen sterilized rat. *Mol. Brain Res.* 20: 71-78.
- Heijna M.H., Padt M., Hogenboom F., Schoffelmeer A.N.M., Mulder A.H. (1991) Opioidreceptor mediated inhibition of (³H) dopamine but not (³H) noradrenaline release from rat mediobasal hypothalamus slices. *Neuroendocrinology* 54: 118-126.
- Horvath T.L., Naftolin F., Leranath C. (1993) Luteinizing hormone-releasing hormone and gamma-aminobutyric acid neurons in the medial preoptic area are synaptic targets of dopamine axons originating in the anterior periventricular areas. *J. Neuroendocrinol.* 5: 71-79.
- Imperato A., Angelucci L., Casolini P., Zocchi A., Puglisi-Allegra S. (1992) Repeated stressful experiences differently affect limbic dopamine release during and following stress. *Brain Res.* 577: 194-199.
- Johnson M.D., Crowley R.W. (1984) Effect of opiate antagonists on serotonin turnover and luteinizing hormone and prolactin secretion in estrogen or morphin treated rats. *Neuroendocrinology* 38: 322-327.
- Kalra S.P., Kalra P.S. (1983) Neural regulation of luteinizing hormone secretion in the rat. *Endocrinol. Rev.* 4: 311-351.
- Kalra S.P., Simpkins J.W. (1981) Evidence for noradrenergic mediation of opioid effects on luteinizing hormone secretion. *Endocrinology* 109: 776-782.
- Kiss J., Halasz B. (1985) Demonstration of serotonergic axons terminating on luteinizing hormone-releasing hormone neurons in the preoptic area of the rat using combination of immunocytochemistry and high resolution autoradiography. *Neuroscience* 14: 69-78.
- Köves K., Marton J., Molnar J., Halasz B. (1981) (D-Met 2 Pro 5)-enkephalinamide - induced blockade of ovulation and its reversal by naloxone in the rat. *Neuroendocrinology* 32: 82-86.
- Kuljis R.O., Advis J.P. (1989) Immunocytochemical and physiological evidence of synapse between dopamine- and luteinizing hormone releasing hormone containing neurons in the ewe median eminence. *Endocrinology* 124: 1579-1582.
- Leadem C.A., Kalra S.P. (1985) Reversal of β -endorphin induced blockade of ovulation and LH surge with prostaglandin E₂. *Endocrinology* 117: 684-689.
- Leranath C., MacLusky N.J., Shanabrough M., Naftolin F. (1988) Catecholaminergic innervation of luteinizing hormone-releasing hormone and glutamic acid decarboxylase immunopositive neurons in the rat medial preoptic area. *Neuroendocrinology* 48: 591-602.
- Levicky J., Dunn A.J. (1993) Corticotropin-releasing factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freely moving rats as assessed by microdialysis. *J. Neurochem.* 60: 602-612.
- Lohse M., Wuttke W. (1981) Release and synthesis rates of catecholamines in hypothalamic, limbic and midbrain structures following intraventricular injection of β -endorphin in male rats. *Brain Res.* 229: 398-402.
- MacLusky N.J., Naftolin F., Leranath C. (1988) Immunocytochemical evidence for direct synaptic connection between corticotropin releasing factor (CRF) and gonadotropin releasing hormone (GnRH) containing neurons in the preoptic area of the rat. *Brain Res.* 471: 391-395.
- Malven P.V., Stanisiewski E.P., Haglof S.A. (1990) Ovine brain areas sensitive to naloxone induced stimulation of luteinizing hormone release. *Neuroendocrinology* 52: 373-381.
- Meyer S.L., Goodman R.L. (1985) Neurotransmitters involved in mediating the steroid - dependent suppression of pulsatile luteinizing hormone secretion in anestrus ewes: Effects of receptor antagonists. *Endocrinology* 116: 2059-2061.
- Meyer S.L., Goodman R.L. (1986) Separate neural systems mediate the steroid - dependent and steroid - independent suppression of tonic luteinizing hormone secretion in the anestrus ewe. *Biol. Reprod.* 35: 563-571.
- Naylor A.M., Porter D.W.F., Lincoln D.W. (1990) Central administration of corticotropin-releasing factor in the sheep: effect on secretion of gonadotropins, prolactin and cortisol. *J. Endocrinol.* 124: 117-125.
- Pacak K., Palkovits M., Kopin I.J., Goldstein D.S. (1995) Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathetic activity: in vivo microdialysis studies. *Front. Neuroendocrinol.* 16: 89-150.
- Pol O., Campmany L., Armario A. (1995) Inhibition of catecholamine synthesis with α -methyl-p-tyrosine apparently increases brain serotonergic activity in the rat: no influence of previous chronic immobilization stress. *Pharmacol. Biochem. Behav.* 52: 107-112.
- Przekop F., Mateusiak K., Stupnicka E., Romanowicz K., Domański E. (1990) Suppressive effect of β -endorphin and naloxone on the secretion of cortisol under stress condition in sheep. *Exp. Clin. Endocrinol.* 95: 307-314.
- Rasmussen D.D. (1991) Dopamine - opioid interaction in the regulation of hypothalamic gonadotrophin releasing hormone (GnRH) secretion. *Neuroendocrinol. Lett.* 13: 419-424.
- Rasmussen D.D., Kennedy B.P., Ziegler M.G., Nett T.M. (1988) Endogenous opioid inhibition and facilitation of gonadotropin-releasing hormone release from the median in vitro: potential role of catecholamines. *Endocrinology* 123: 2916-2921.
- Reymond M.J., Kaur C., Porter J.C. (1983) Inhibitory role for morphine on the release of dopamine into hypophysial portal blood and on the synthesis of dopamine in tuberoinfundibular neurons. *Brain Res.* 262: 253-258.

- Rivest S., Rivier C. (1995) The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive functions. *Endocrinol. Rev.* 16: 177-199.
- Shimuzu N., Nakane H., Hori T., Hayashi Y. (1994) CRF receptor antagonist attenuates stress-induced noradrenaline release in the medial prefrontal cortex of rats. *Brain Res.* 654: 145-148.
- Thind K.K., Goldsmith P.C. (1988) Infundibular gonadotropin-releasing hormone neurons are inhibited by direct opioid and autoregulatory synapses in juvenile monkeys. *Neuroendocrinology* 47: 203-216.
- Tomaszewska D., Mateusiak K., Przekop F. (1992) Changes in extracellular monoamines in the nucleus infundibularis-median eminence in ewes during estrus: response to CRF. Ninth International Congress of Endocrinology, Nicea, Abstracts, p.557.
- Tomaszewska D., Mateusiak K., Przekop F. (1994) Alterations in extracellular levels of monoamines and their metabolites in the MBH-ME of estrous ewes during β -endorphin treatment. 3-rd International Congress of Neuroendocrinology, Budapest, Abstracts, p. 72.
- Tomaszewska D., Przekop F., Mateusiak K. (1995) The influence of naloxone and CRF antagonist on monoaminergic systems activity in the mediobasal hypothalamus-median eminence of ewes in normal and stressful condition. Six Symposium of Catecholamines and Other Neurotransmitters in Stress. Smolenice Castle, Abstracts, p. 88.
- Vetrugno G.C., Lachner J., Perego C., Miromda E., De Simoni M.G., Tappaz M. (1993) Lack of glucocorticoids sustains the stress-induced release of noradrenaline in the anterior hypothalamus. *Neuroendocrinology* 57: 835-842.
- Webster E.L., Grigoriadis D.E. (1991) Corticotropin-releasing factor receptors in the brain-pituitary-immune axis. In: Stress, neuropeptides and systemic disease (Eds. J.A. McCubbin, P.G. Komfman and C.B. Nemeroff). Academic Press Inc., San Diego, p. 233-260.
- Weesner G.D., Malven P.V. (1990) Intracerebral immunoneutralization of β -endorphin and Met-Enkephalin disinhibits release of pituitary luteinizing hormone in sheep. *Neuroendocrinology* 52: 382-388.
- Whisnant C.S., Havern R.L., Goodman R.L. (1991) Endogenous opioid suppression of luteinizing hormone pulse frequency and amplitude in the ewe - hypothalamic sites of action. *Neuroendocrinology* 54: 587-593.
- Zetterström T., Sharp T., Collin A.K., Ungerstedt U. (1988) In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine - releasing drugs: implications for origin of extracellular DOPAC. *Eur. J. Pharmacol.* 148: 327-334.

Received 10 April 1996, accepted 15 May 1996