Journal of Animal and Feed Sciences, 13, 2004, 121–132

The role of $GABA_A$ receptors in the neural systems of the medial preoptic area in the control of GnRH release during the luteal phase of the oestrous cycle in ewes

D. Tomaszewska-Zaremba[1], K. Mateusiak and F. Przekop

The Kielanowski Institute of Animal Physiology and Nutrition,
Polish Academy of Sciences
05-110 Jabłonna, Poland

(Received 2 October 2003; accepted 14 January 2004)

ABSTRACT

Recent studies from our laboratory suggested that functional interconnection of the GABAergic system with the GnRHergic, β-endorphinergic and catecholaminergic systems in the medial preoptic area (MPOA) plays an important role in the modulation of gonadoliberin (GnRH) release during the follicular phase of the oestrous cycle in ewes. Since the mode of action of γ-aminobutyric acid (GABA) on GnRH neurons is highly dependent on gonadal steroids, we extended our studies to the role of $GABA_A$ receptors in the MPOA on the activity of all of the above-mentioned systems during the luteal phase of the oestrous cycle. Stimulation of $GABA_A$ receptors by muscimol did not affect either GnRH/LH, β-endorphin release or catecholaminergic activity, expressed by extracellular concentrations of noradrenaline (NE), dopamine (DA) and their metabolites MHPG and DOPAC. Blockade of $GABA_A$ receptors decreased β-endorphin release, dopaminergic and noradrenergic activity but did not change GnRH release or LH secretion. It is suggested that progesterone secretion during the luteal phase may desensitize $GABA_A$ receptors to further stimulation.

KEY WORDS: ewe, hypothalamus, gonadotropin-releasing hormone (GnRH), β-endorphin (B-END), γ-aminobutyric acid (GABA), catecholamines

[1] Corresponding author: e-mail: d_tomaszewska@poczta.onet.pl

INTRODUCTION

Neuroendocrine control of GnRH/LH release involves both excitatory and inhibitory components. Among these transsynaptic regulatory systems, γ-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the hypothalamus (Decavel and van den Pol, 1990) and preoptic area (Robinson, 1995, 1997), affects gonadoliberin (GnRH) release by two classes of membrane receptors: $GABA_A$ (Sieghart, 1995) and $GABA_B$ (Mott and Lewis, 1994). Numerous experiments indicate that GABA mediates both inhibition (Scott and Clarke, 1993; Leonhardt et al., 1995) as well as stimulation (Jarry et al., 1992; Leonhardt et al., 2000) of GnRH release by $GABA_A$ receptor mechanism(s). The dual inhibitory-stimulatory action of GABA on GnRH release is not clearly understood. GABA may inhibit or stimulate GnRH release in the hypothalamus in at least two ways: first, directly by a $GABA_A$ receptor mechanism on GnRH neurons (Petersen et al., 1993; Jung et al., 1998) and second, through $GABA_A$ receptor processes on inhibitory or stimulatory interneurons that impinge on GnRH cells (Horvath et al., 1992, 1993). Indeed, our previous results strongly suggest that functional interconnection of GABAergic neurons with β-endorphinergic and dopaminergic systems in the ventromedial hypothalamus-nucleus infundibularis (VEN/NI) (Tomaszewska-Zaremba et al., 2003a) and in the preoptic area (MPOA) (Tomaszewska-Zaremba et al., 2003b) plays an important role in the control of GnRH release during the follicular phase of the oestrous cycle. Since GnRH neuronal activity and function of the β-endorphinergic (Domański et al., 1991; Conover et al., 1993), noradrenergic and dopaminergic (Chomicka et al., 1994) systems in the control of GnRH release depend on gonadal hormone concentrations and changes in various phases of the reproductive cycle, it is reasonable to suggest that the influence of GABA on these systems varies during the time course of the oestrous cycle. This assumption prompted us to investigate the effect of a $GABA_A$ receptor agonist, muscimol, and antagonist, bicuculline, on GnRH and β-endorphin (B-END) release, and on catecholaminergic activity in the MPOA of ewes during the luteal phase of the oestrous cycle.

MATERIAL AND METHODS

Animals

The studies were performed on four-year-old Polish Merino ewes, well adapted to the experimental conditions. Using a stereotaxic procedure (Traczyk and Przekop, 1963; Welento et al., 1969), permanent guide cannulae were positioned on the skull at least three weeks prior to the perfusion procedure. The surgical opera-

tion of ewes was carried out under Vetbutal (Biovet, Puławy, Poland) anaesthesia. The guide cannulae were directed towards the MPOA by a stereotaxic procedure and were secured to the skull with screws and dental cement. Throughout the study the animals were maintained indoors at a temperature of 12-15°C in individual pens and exposed to natural daylight. They always had visual contact with their neighbours, even during the sampling periods, to prevent the stress of social isolation.

Feed and water were available *ad libitum*. On the day of experiments two hours prior to perfusion, push-pull cannulae were introduced through the guide cannulae and placed in the proper position according to coordinates for the ovine hypothalamus. Each animal received two perfusions: first, a control perfusion with Ringer solution on the 7-th day of the oestrous cycle, and a second perfusion with muscimol (10 μg muscimol/ml Ringer solution) or bicuculline (30 μg bicuculline/ml Ringer solution) on the 7-th day of the next oestrous cycle. Each ewe served as a control. After the experiments the animals were euthanised with a barbiturate overdose. The procedures were done with the consent of the Local Ethical Committee at Warsaw University of Agriculture. All experiments were performed during September-November.

Perfusate collection

The perfusions were performed at a flow rate of 5 μl/min and perfusates were collected continuously for 5 h (9.00-14.00) in 30-min fractions into tubes containing 50 μl 0.1 mM ascorbic acid. The perfusates were kept in an ice bath during sampling and stored at -80°C until assay. To determine the site of perfusion and to localize the place from which the perfusates were sampled, the brain of each animal was infused with 20 μl Prussian blue for 10 min. Then the brains were removed and sectioned sagitally under a stereoscopic binocular. Stained tissue was established in a spherical fraction about 2.0-2.5 mm around the tip of the cannula. The perfusates of animals with misplaced cannulae were excluded from analysis.

Analysis of catecholamines and their metabolites

Catecholaminergic system activity was evaluated on the basis of the extracellular concentration of noradrenaline (NE), dopamine (DA) and their main metabolites: 4-hydroxy-3-methoxy-phenylglycol (MHPG), 3,4-dihydroxy-phenylacetic acid (DOPAC). The concentrations of these compounds were analysed using high performance liquid chromatography with electrochemical detection as described previously (Tomaszewska-Zaremba et al., 2003). The limit of detection was 4 pg/50 μl for NE, 5 pg/50 μl for DA 3 pg/50 μl for MHPG and 3 pg/50 μl for DOPAC.

Analysis of β-endorphin in perfusates

Extraction of β-endorphin-like immunoreactivity compound(s) was performed by a method similar to the procedure described by Leshin and Malven (1984) and modified as described previously (Tomaszewska-Zaremba et al., 2003). β-endorphin antibodies were kindly provided by G. Martin from The University of West Australia, Needlands. The detection limit was 6 pg/50 µl. The recovery of β-endorphin-like immunoreactivity calculated on the basis of extraction using ^{125}Iβ-endorphin added to plasma samples was 85-87%. Intra- and interassay coefficients of variation were 11 and 16%, respectively.

Radioimmunoassay of GnRH

The RIA procedure for GnRH was similar to that previously reported (Domański et al., 1991). The reference GnRH was obtained from Sigma. GnRH was radioiodinated using the chloramine-T method. GnRH antibodies (kindly provided by J. Kosowicz, Medical School, Poznań, Poland) at a final dilution of 1:16000 were used for determination. The detection limit was 1.9 pg/100 µl. The intra- and interassay coefficients were 7 and 11%, respectively.

Radioimmunoassay of LH

Plasma LH concentration was assayed by a double-antibody RIA using antiovine - LH and antirabbit - gammaglobulin antisera and ovine LH standard (NIH-LH-SO18) according to Stupnicki and Madej (1976). The assay sensitivity was 0.06 ng/ml and intra- and interassay coefficients of variation were 4 and 10%, respectively.

Data analysis

Control levels of NE, DA, MHPG and DOPAC in the MPOA were evaluated in perfusates collected from Ringer-solution-treated animals. The effect of perfusion of muscimol or bicuculline into the MPOA on the extracellular concentration of these compound(s) was expressed as the percent change in the respective fraction pairs of muscimol or bicuculline to control animals, respectively. All results are presented as mean ± SEM. One-way ANOVA was used followed by Tukey's test to evaluate differences between means of concentrations in the respective pair fractions in perfusates from muscimol- or bicuculline-treated animals to controls.

B-END concentrations are expressed as means ± SEM and were assessed by one-way ANOVA following by Tukey's test. GnRH concentrations are expressed as means ± SEM and were assessed by one-way ANOVA, differences between

muscimol or bicuculline and the control treatment in particular pair fractions were evaluated by Tukey's test.

Plasma LH concentrations are expressed as a mean ± SEM. The significance of differences between the results of control and muscimol or bicuculline treatment was assessed by ANOVA followed by the least significant differences (LSD) test (STATISTICA). The number of LH pulses and amplitude was determined by the PC-PULSAR computer program according to the method of Marriam and Wachter (1982) with G parameters: G1 = 3.98; G2 = 2.40; G3 = 1.68, G4 = 1.24 and G5 = 0.93. The frequency in controls and muscimol- or bicuculline- treated animals was defined as the number of identified pulses per collecting period and is expressed as a mean ± SEM. The amplitude was defined as the difference between peak and nadir values (Viguie et al., 1995). Differences in LH pulse frequency and amplitude in the vehicle and muscimol or bicuculline perfused groups were analysed by the Wilcoxon test.

RESULTS

Effects of muscimol and bicuculline perfusion in the MPOA on the extracellular concentration of GnRH in this structure and LH level in blood plasma

A schematic diagram showing the location of the perfusion sites in the MPOA is presented in Figure 1. The concentration of GnRH in the control perfusates was low and in some samples the value of this hormone was at, or near the assay sensitivity limit. Muscimol perfusion did change neither the GnRH concentration in perfusates (Figure 2a) nor LH pulse frequency, pulse amplitude and LH level

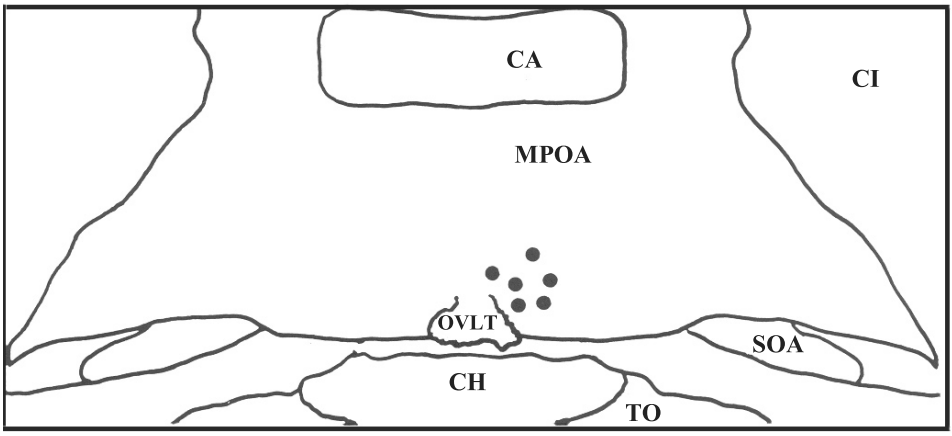

Figure 1. Frontal section through the preoptic area showing location of muscimol perfusion (circles). CA-commissura anterior, CH- chiasma opticum, CI- capsula interna, MPOA- medial preoptic area, OVLT- organum vasculosum of the lamina terminalis, SOA- supraoptic nucleus, TO- tractus opticus

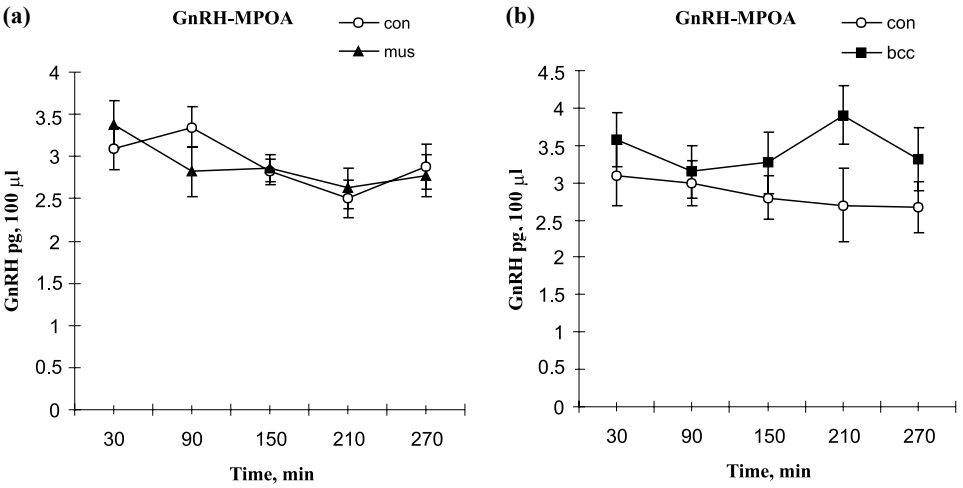

Figure 2. The effect of muscimol (a) and bicuculline (b) perfusion into MPOA on GnRH concentrations in perfusates. Data presented are mean value±SEM (n=6, *P≤0.05, **P≤0.01)

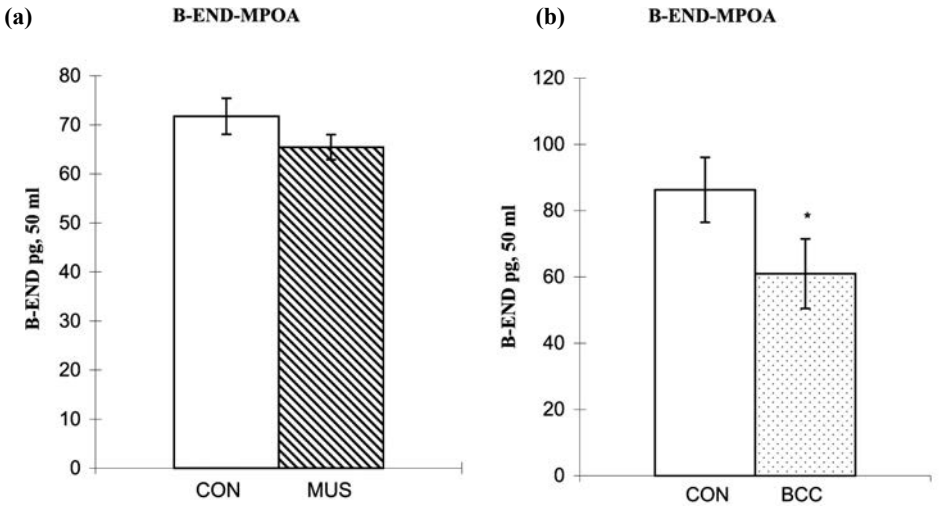

Figure 3. The effect of muscimol (a) and bicuculline (b) perfusion into MPOA on the β-endorphin concentrations in perfusates. Data presented are mean value ± SEM (n=6, *P≤0.05, **P≤0.01)

in blood plasma (data not shown). In all animals after muscimol perfusion the oestrous cycle occurred at the physiological time.

Perfusion with bicuculline in the MPOA did not cause evident changes in the extracellular concentration of GnRH in all animals (Figure 2b). Bicuculline did not change either LH concentration in blood plasma or the pattern of this hormone's secretion, i.e. the pulse amplitude and frequency.

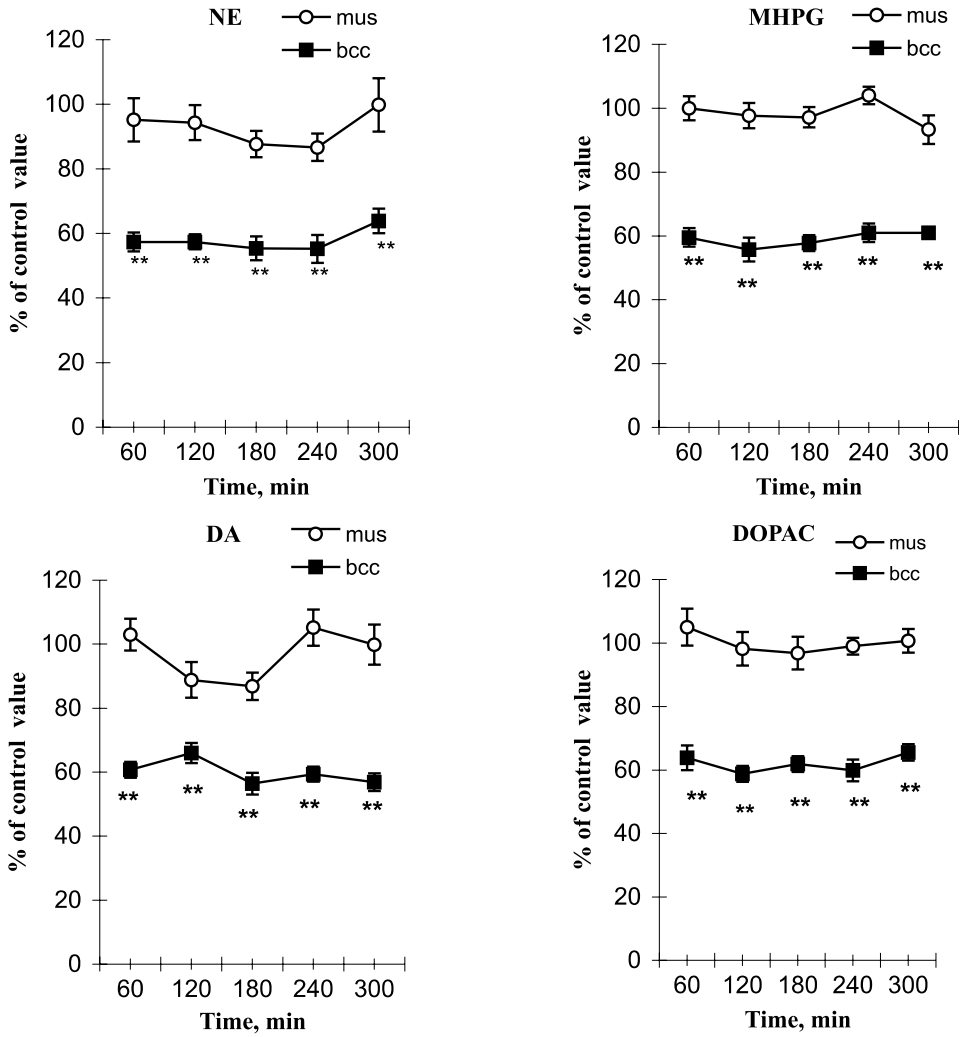

Figure 4. Extracellular concentrations of NE, DA, MHPG and DOPAC in MPOA of ewes during perfusion of muscimol and bicuculline. Data are expressed as a percent of the control values in the respective pair fractions (muscimol/bicuculline treatment to Ringer solution treatment), data presented are mean values ± SEM (n=6, *P≤0.05, **P≤0.01)

Effects of muscimol and bicuculline perfusion in the MPOA on the extracellular concentration of B-END-LI in perfusates

During the luteal phase of the oestrous cycle in ewes, the extracellular concentration of B-END-LI in this structure varied markedly. Analysis of the response magnitude to muscimol in individual ewes showed that only in one animal did the decline of the B-END-LI concentration reach statistical significance as compared with controls.

The mean concentration of B-END-LI in perfusates in the muscimol-treated animals therefore did not differ significantly as compared with levels in these ewes during vehicle perfusion (Figure 3a). Analysis of the magnitude of the β-endorphin response to bicuculline treatment in individual ewes indicated that blockade of $GABA_A$ receptors failed to change this opioid concentration in 2 animals; in the remaining 4 ewes bicuculline significantly decreased B-END-LI levels in perfusates. The overall mean concentration of B-END-LI in perfusates collected during bicuculline treatment differed significantly from controls (Figure 3b).

Effect of muscimol and bicuculline perfusion in the MPOA on the extracellular concentration of NE, DA, MHPG, DOPAC

In the control treatment the concentrations of NE and DA in perfusates ranged from 6 to 42 pg/50 µl and from 15 to 104 pg/50 µl, respectively, depending on the individual ewe. The concentrations of MHPG in perfusates ranged from 27 to 276 pg/50 µl and DOPAC from 55 to 383 pg/50 µl in individual animals. Perfusion of muscimol did not cause a marked change in extracellular concentrations of catecholamines or their metabolites (Figure 4).

In all animals bicuculline treatment significantly depressed the extracellular concentrations of NE, DA and their metabolites, MHPG and DOPAC (Figure 4).

DISCUSSION

These data show that perfusion of muscimol into the MPOA of ewes during the luteal phase of the oestrous cycle did not significantly alter either GnRH or β-endorphin release or catecholaminergic activity in this structure. Lack of changes in the pattern of LH secretion strongly suggests that the $GABA_A$ receptor agonist in the MPOA did not alter GnRH release into the VEN/NI. This point of view is supported by our previous findings showing that muscimol perfusion in the VEN/NI apparently did not affect GnRH/LH secretion in ewes during the luteal phase of the oestrous cycle (Tomaszewska-Zaremba et al., 2002). Since blockade of $GABA_A$ receptors in the MPOA of ewes during the luteal phase of the oestrous cycle suppressed both β-endorphin release and catecholaminergic system activity, the failure of muscimol to alter activity of these systems is not likely a reflection of either a minor or no role for $GABA_A$ receptors in the MPOA in modulating GnRH, β-endorphin, NE and DA release. The dose of muscimol was chosen according to the literature (Scott and Clarke, 1993) and on the basis of our earlier works. Higher doses of muscimol caused toxic symptoms (convulsions, salivate). In this setting, one plausible explanation for lack of response of these systems to the $GABA_A$ receptor agonist would be that the progesterone-induced $GABA_A$ release during the luteal phase may desensitize $GABA_A$ receptors and make them resistant to further stimulation. Indeed, recent works have revealed a

clear stimulatory effect of progesterone on GABA release in the medial preoptic area (Robinson and Kendrick, 1992; Robinson, 1995). In the light of these results it is reasonable to suggest that progesterone secreted during the luteal phase of the oestrous cycle may affect the dynamics of GnRH release through a influence on GABA release or indirectly by changes of β-endorphinergic and catecholaminergic system activities. In such multifactoral actions of GABA on gonadotropin secretion it is not possible to distinguish in detail the action of the GABAergic system in the control of GnRH release. This is particularly so because the applied method did not enable analysis of the direct influence of muscimol on the activity of GnRH neurons.

Blockade of $GABA_A$ receptors may also affect in different ways the mechanism(s) controlling GnRH release. The presented results indicate that bicuculline reduced the tone of β-endorphinergic activity but did not increase GnRH release; such event is not strange because it is well documented that the inhibitory effects of β-endorphin on GnRH release are only marginally apparent in the luteal phase of the oestrous cycle in ewes (Conover et al., 1993). The inhibitory effect of β-endorphin on GnRH/LH release (Curlevis et al., 1991; Domański et al., 1991; Conover et al., 1993) as well as the stimulatory influence of naloxone (Conover et al., 1993) on GnRH secretion in the VEN/NI in ewes increases distinctly throughout the follicular phase reaching the highest value just prior to the preovulatory surge of gonadotropin. However, at the MPOA of ovariectomized-progesterone treated ewes the decrease of opioidergic activity by naltrexon suppressed the stimulatory influence of progesterone on GABA release and enhanced LH secretion (Tartonese, 1999). Our present findings together with those of previous reports support the notion that the specific actions of β-endorphin on GnRH/LH secretion is dependent upon the ovarian hormones concentration.

Temporal analysis of the relationship between bicuculline treatment and extracellular concentrations of DA and DOPAC indicated that the $GABA_A$ receptor antagonist decreases dopaminergic activity in the MPOA and that dopamine does not play an important role in this structure on GnRH release during the luteal phase of the oestrous cycle. The role of dopamine in the regulation of GnRH release is still not consistent to provide a clear understanding of its action in this process.

The increased dopaminergic activity in the VEN/NI during the luteal phase suggests an inhibitory effect of DA on GnRH release (Chomicka et al., 1994); the other results rise the possibility that the dopaminergic system in this structure is part of the neural pathways mediating the positive feedback effects of oestradiol on GnRH secretion (Andersen et al., 2001). On the other hand, in the MPOA the progesterone-induced increase of GABA secretion did not change DA release, thus suggesting that the GABA-induced suppression of LH release cannot be attributed to increased dopaminergic system activity in this structure (Robinson et al., 1991). Similarly, lack of changes in GnRH/LH release with a concomitant decrease of noradrenergic

system activity during the luteal phase under bicuculline treatment indicates that suppression of NE release has a rather minor role in GnRH release in the MPOA. The physiological significance of NE in the control of gonadotropin secretion is still not unequivocally established. Most of the results implicating noradrenaline in the regulation of GnRH release suggest the MPOA as the main site of the stimulatory influence of the noradrenergic system on gonadotropin secretion (Robinson et al 1991; Robinson and Kendrick, 1992); however, the mode of action of noradrenaline on GnRH release seems highly dependent on the influence of season and steroid hormone concentration (Scott et al., 1992; Goodman, 1996).

CONCLUSIONS

In conclusion, these results indicate that stimulation of $GABA_A$ receptors does not affect either GnRH, β-endorphin release or catecholaminergic activity. Blockade of these receptors did not change GnRH release, although it reduced β-endorphinergic and catecholaminergic activity. It is suggested that the increased concentration of progesterone during the luteal phase may desensitize $GABA_A$ receptors to further stimulation. Further studies are needed to explain the lack of changes in GnRH release under blockade of $GABA_A$ receptors leading to the decrease of β-endorphinergic and catecholaminergic system activity.

REFERENCES

Andersen S.T., Walsh J.P., Tillet Y., Clarke I.J., Curlevis J.D., 2001. Dopaminergic input to the ventromedial hypothalamus facilitates the estrogen-induced luteinizing hormone surge in ewes. Neuroendocrinology 73, 91-101

Chomicka L.K., Wolińska-Witort E., Przekop F., 1994. Release of LHRH, β-endorphin and dopamine by the nucleus infundibularis-median eminence during the estrous cycle in the ewe. IIIrd European Congress of Endocrinology, Amsterdam. Eur. J. Endocrinol. 130, Suppl. 2, 43

Conover C.D., Kuljis R.O., Rabii J., Advis J.P., 1993. β-endorphin regulation of luteinizing hormon-releasing hormone release 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. Effect of β-endorphin on pulsatile luteinizing hormone and prolactin secretion during the follicular phase in the ewe. J. Neuroendocrinol. 3, 123-126

Decavel C., van den Pol A.N., 1990. GABA: a dominant neurotransmitter in the hypothalamus. J. Comp. Neurol. 302, 1019-1037

Domański E., Chomicka L.K., Ostrowska A., Gajewska 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

Goodman R.L., 1996. Neural systems mediating the negative feedback actions of estradiol and progesterone in the ewe. Acta Neurobiol. Exp. 56, 727-741

Herbison A.E., 1998. Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. Endocrine Rev. 19, 302-330

Horvath T.L., Naftolin F., Leranth C., 1992. Gabaergic and catecholaminergic innervation of mediobasal hypothalamic β-endorphin cell projecting to the medial preoptic area. Neuroscience 51, 391-399

Horvath T.L., Naftolin F., Leranth C., 1993. Luteinizing hormone-releasing hormone and gamma-aminobutyric acid neurons in the medial preoptic area are synaptic targets of dopamine axons originating in anterior periventricular areas. J. Neuroendocrinol. 5, 71-79

Jarry H., Leonhardt S., Schwarz M., Wuttke W., 1992. Amino acid neurotransmitter release in the preoptic area of rats during the positive feedback actions of estradiol on LH release. Neuroendocrinology 56, 133-140

Jung H., Shannon E.M., Fritschy J-M., Ojeda S.R., 1998. Several GABA receptor subunits are expressed in LHRH neurons of juvenile female rats. Brain Res. 790, 219-229

Leonhardt S., Boning B., Luft H., Wuttke W., Jarry H., 2000. Activation of gene expression of the γ-aminobutyric acid rather than the glutamatergic system in the preoptic area during the preovulatory gonadotropin surge of the rat. Neuroendocrinology 71, 8-15

Leonhardt S., Seong J.Y., Kim K., Thorun Y., Wuttke W., 1995. Activation of central GABA-A but not GABA-B receptors rapidly reduces pituitary LH release and GnRH gene expression in the preoptic/ anterior hypothalamic area of ovariectomized rats. Neuroendocrinology 61, 655-662

Leshin L.S., Malven P.V., 1984. Radioimmunoassay for β-endorphin (lipotropin) in unextracted plasma from sheep. Domes. Anim. Endocrinol. 1, 175-188

Merriam G.R., Wachter K.W., 1982. Algorithms for the study of episodic hormone secretion. Amer. J. Physiol.-Endocrinol. Met. 243 (6), E310-E318

Mott D.D., Lewis D.V., 1994. The pharmacology and function of central $GABA_B$ receptors. Int. Rev. Neurobiol. 36, 97-223

Petersen J.E., McCrone S., Coy D., Adelman J.P., Mahom L.C., 1993. $GABA_A$ receptor subunit mRNA in cells of preoptic area: colocalization with LHRH mRNA using dual-label in situ hybridization histochemistry. Endocrinol. J. 1, 29-34

Robinson J.E., Kendrick K.M., Lambart C.E., 1991. Changes in the release of gamma-aminobutyric acid and catecholamines in the preoptic area prior to and during the preovulatory surge of luteinizing hormone in ewe. J. Neuroendocrinol. 3, 393-399

Robinson J.E., 1995. Gamma-aminobutyric acid and the control of GnRH secretion in sheep. J. Reprod. Fertil., Suppl. 49, 221-230

Robinson J.E., Kendrick K.M., 1992. Inhibition of luteinizing hormone secretion in the ewe by progesterone: associated changes in the release of gamma-aminobutyric acid and noradrenaline in the preoptic area as measured by intracranial microdialysis. J. Neuroendocrinol. 4, 231-236

Scott C.J., Clarke I.J., 1993. Evidence that changes in the function of the subtypes of the receptors for γ-aminobutyric acid may be involved in the seasonal changes in the negative-feedback effects of estrogen on gonadotropin-releasing hormone secretion and plasma luteinizing hormone levels in the ewe. Endocrinology 133, 2904-2912

Scott C.J., Cummins J.T., Clarke I.J., 1992. Effect on plasma luteinizing hormone levels of microinjection of noradrenaline and adrenaline into the septo-preoptic area of the brain of the ovariectomized ewe: changes with season and chronic estrogen treatment. J. Neuroendocrinol. 4, 131-141

Sieghart W., 1995. Structure and pharmacology of gamma-aminobutyric $acid_A$ receptor subtypes. Pharmacol. Rev. 47, 181-234

Stupnicki R., Madej A., 1976. Radioimmunoassay of LH in blood plasma of farm animals. Endocrinologie 68, 6-13

Tartonese D.J., 1999. Interaction between hypothalamic systems in the photoperiodic regulation of pulsatile luteinizing hormone secretion in sheep. Endocrinology 140, 750-757

Tomaszewska-Zaremba D., Mateusiak K., Przekop F., 2003a. The role of $GABA_A$ receptors in the neural systems of the ventromedial hypothalamus-nucleus infundibular region in the control of GnRH release in ewes during follicular phase. Exp. Clin. Endocrinol. Diabetes 111, 335-340

Tomaszewska-Zaremba D., Przekop F., Mateusiak K., 2003b. The role of $GABA_A$ receptors in the neural systems of medial preoptic area in the control of GnRH release in ewes during follicular phase. Anim. Reprod. Sci. 77, 71-83

Tomaszewska-Zaremba D., Przekop F., Mateusiak K., 2002. The role of $GABA_A$ receptors in the neural systems of the ventromedial-infundibular region of the hypothalamus in the control of gonadoliberin release during luteal phase in ewes. J. Physiol. Pharmacol. 53, 835-845

Traczyk W., Przekop F., 1963. Methods of investigation of the function of hypothalamus and hypophysis in chronic experiments in sheep. Acta Physiol. Pol. 14, 217-226

Viguie C., Caraty A., Locatelli A., Malpaux B., 1995. Regulation of luteinizing hormone-releasing hormone (LHRH) secretion by melatonin in the ewe. Simultaneous delayed increase in LHRH and luteinizing hormone pulsatile secretion. Biol. Reprod. 52, 1114-1120

Welento J., Steyn S., Milart Z., 1969. Observation on the stereotaxic configuration of the hypothalamus nuclei in sheep. Anat. Anz. 124, 1-27

STRESZCZENIE

Rola receptorów $GABA_A$ w neuralnej regulacji uwalniania GnRH z okolicy przedwzrokowej u owiec w fazie lutealnej cyklu estralnego

Wcześniejsze badania wykonane w naszym laboratorium sugerują, że funkcjonalne połączenie układu GABA-ergicznego z układami gonadoliberyny, β-endorfinergicznym i katecholaminergicznym w okolicy przedwzrokowej odgrywa ważną rolę w modulacji uwalniania GnRH w czasie fazy pęcherzykowej cyklu estralnego u owiec. Działanie GABA na neurony GnRH w dużym stopniu zależy od poziomu steroidów gonadowych, dlatego rozszerzyliśmy nasze badania nad rolą receptorów $GABA_A$ w regulacji w/w układów w MPOA na fazę lutealną cyklu estralnego. Stymulacja receptorów $GABA_A$ muscimolem nie wpływała zarówno na uwalnianie GnRH/LH i β-endorfiny jak i na aktywność układów katecholaminergicznych. Blokada receptorów $GABA_A$ poprzez podanie bicuculliny obniżała uwalnianie β-endorfiny, zmniejszała aktywność układów dopaminergicznego i noradrenergicznego, natomiast nie zmieniała sekrecji GnRH/LH. Sugeruje się, że w czasie fazy lutealnej cyklu estralnego indukowany progesteronem wzrost aktywności układu GABA-ergicznego czyni receptor $GABA_A$ mało wrażliwym na dalszą stymulację muscimolem.