

Review

Control of pulsatile LH secretion during seasonal anoestrus in the ewe

Jaime Gallegos-Sánchez*, Benoit Malpaux, Jean-Claude Thiéry**

Station de physiologie des mammifères domestiques, Inra, 37380 Nouzilly, France

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Abstract – The seasonality of reproductive activity in the ewe in temperate latitudes is controlled by photoperiod. Its annual variations control the temporal organization of the sexual cycle by changing the activity of the gonadotrophic axis. Cyclic oestrous behaviour usually appears in the ewe at the end of summer or the beginning of autumn and finishes in winter or at the very beginning of spring. Seasonal anoestrus is characterized by the absence of ovulation and sexual behaviour. During seasonal anoestrus, a decrease in LH pulse frequency is observed. The inhibition of pulsatile LH secretion is maintained throughout the anoestrous season and is responsible for the low reproductive activity during this period. Variation in the seasonal inhibition of LH pulsatility results from an increase in the negative feedback by oestradiol on LH pulse frequency during the long days of spring and summer. The inhibition of LH secretion involves increased action of dopamine in the hypothalamus on the chain of nervous elements which controls gonadotrophic activity. Among the various dopaminergic structures, the retrochiasmatic A15 nucleus is involved in the inhibitory control of LH pulsatility by oestradiol during the long day period. Oestradiol increases the dopaminergic tone of the A15 nucleus in ovariectomized ewes during the long day period. In this structure, the effect of oestradiol on the dopaminergic metabolism probably results from a direct, local activation. In the sheep, dopamine might also participate in the inhibition of gonadotrophin activity during other periods of reproductive life. © Inra/Elsevier, Paris

anoestrus / ewe / hypothalamus / LH pulses / dopamine

Résumé – **Contrôle de la sécrétion pulsatile de LH pendant l'anoestrus saisonnier chez la brebis.** Chez la brebis en climat tempéré, l'activité de reproduction est contrôlée par la photopériode, dont les variations annuelles sont responsables des variations d'activité de l'axe gonadotrope hypothalamo-hypophysio-gonadique. Les cycles œstriens de la brebis apparaissent à la fin de l'été et en automne et se terminent à la fin de l'hiver ou au début du printemps. Pendant l'anoestrus saisonnier, il n'y a pas de comportement d'œstrus ni d'ovulation. Durant cette période (jours longs), la sécrétion pulsatile de LH diminue et se maintient à un niveau de fréquence

* Current address: Colegio de Postgraduados, Programa de Ganaderia, km 35.5 Carretera Mexico-Texcoco, 56230 Montecillo Edo de Mexico, Mexico

** Correspondence and reprints

basse. Cette fréquence basse est le résultat d'une augmentation forte de la rétroaction négative de l'œstradiol sur la sécrétion pulsatile du LHRH hypothalamique. Cet effet implique la dopamine et spécialement le noyau dopaminergique A15 de l'hypothalamus. À ce niveau, l'œstradiol, qui paraît agir localement, stimule l'activité dopaminergique pendant les jours longs. La dopamine paraît également participer à d'autres inhibitions de l'activité gonadotrope chez les ovins. © Inra/Elsevier, Paris

anœstrus / brebis / hypothalamus / dopamine

1. INTRODUCTION

Under temperate latitudes the reproductive function of the ewe involves a series of physiological events at different times throughout the year. The seasonality of reproductive activity in the ewe is controlled by environmental factors [53], mainly photoperiod, which organize the annual sexual cycle [50]. Ewes have a breeding season, characterized by a succession of 16- to 18-day-long oestrous cycles, which usually appears at the end of summer or the beginning of autumn and finishes in winter or at the very beginning of spring [20, 59, 65, 81]. Thereafter follows the anoestrous season, characterized

by the absence of ovulation and sexual behavior [31, 53, 80, 81, 94].

The change in reproductive status is controlled by modifications in the activity of the gonadotrophic axis through variations in the pulsatile LH secretion. During seasonal anoestrus, a low LH pulse frequency (2 pulses \times 24 h⁻¹) is observed. Inhibition of the pulsatile LH secretion is maintained throughout the anoestrous season and is responsible for the low reproductive activity during this period [28, 56, 91]. Similarly, in ovariectomized ewes with a subcutaneous 17 β oestradiol implant, which releases a constant amount of this hormone throughout the year (*figure 1*), LH pulse frequency is reduced dur-

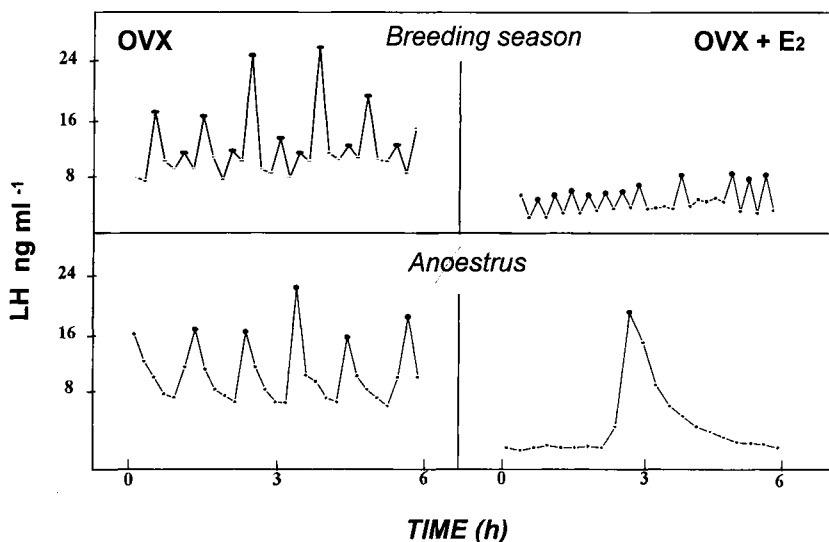


Figure 1. Profiles of plasmatic LH contents observed over 6 h in the model of ovariectomized ewes bearing (OVX + E2) or not (OVX) subcutaneous oestradiol implants. Note the dramatic change in pulsatility in ewes bearing oestradiol implants under anoestrus. Redrawn from Goodman et al. [28].

ing the anoestrus of intact females or during constant long days (16L/8D; [28, 39, 54, 55]). During the breeding season, the oestradiol implant slightly decreases the amplitude of the pulses without significantly modifying the frequency. Thus the variation in the seasonal inhibition of LH pulsatility results mainly from an increase in the negative oestradiol feedback on the LH pulse frequency during the long days of spring and summer [38, 44]. It should also be mentioned that a direct, non-oestradiol-dependent effect of photoperiod on LHRH/LH pulsatility has been clearly demonstrated, but up to now, it appears quantitatively minor [58, 92]. As the secretion of LH is not spontaneously pulsatile but depends on the pulsatile stimulation of pituitary gonadotrophs by the hypothalamic LHRH, the photoperiodic control of sexual activity in the ewe concerns the control of the LHRH neurons which release the neurohormone.

The ewe detects photoperiodic variations through melatonin which is secreted at night. The duration of secretion, then, provides information on day length. This hormone acts in the mediobasal hypothalamus to modulate the reproductive activity [51] but the subsequent nervous pathways involved are still unknown. We do know, however, some of the final steps in the chain of nervous response, between the sites of melatonin action and the LHRH cells, which control the gonadotrophic activity. This review presents an update of our current understanding of these neural connections.

2. NERVOUS REGULATION OF LH RELEASE DURING ANOESTRUS IN THE EWE

2.1. Anatomical structures involved

The immunohistochemical localization of LHRH neurones in the sheep showed

that there were a large number of cell bodies in the septal/preoptic areas of the brain (about 60 % of the total population in the brain; [1, 27, 37]). About 15 % of the LHRH neurons are detected in the mediobasal hypothalamus [11]. These could participate in the basal pulsatile LH secretion. The axons of more than 50 % of the LHRH neurons have terminals in the organum vasculosum of the lamina terminalis (OVLt), the others project to the median eminence where they constitute the integration terminal site for the various sources of incoming data which modulate gonadotrophic secretions. Interestingly, it was shown that the LHRH cells from the preoptic area underwent morphological changes in relation to season. Indeed, there are larger dendritic processes during the anoestrous season [46], and an increase of the innervation of the preoptic LHRH elements during the breeding season [93]. However, the involvement of these changes in relation to the inhibition of LH release during anoestrus remains to be demonstrated.

In the ewe, Domanski et al. [17] and Przekop [63] have shown that lesions in the mediobasal hypothalamus interrupted sexual behaviour and the cyclic activity of the ovaries, while lesions limited to the anterior hypothalamus (AH) can suppress the seasonal anoestrus without blocking the cycle [63]. The identification of monoaminergic structures by immunohistochemical methods also contributed to increasing the understanding of the role of the brain in the seasonality of reproduction in sheep (see Tillet [82] for a review). The anterior part of the hypothalamus, the destruction of which blocks anoestrus contains the retrochiasmatic area where the A15 dopaminergic nucleus is located. In the ovariectomized ewe supplemented with oestradiol, and in the intact ewe during the anoestrous season, the lesions in dopaminergic A15 nuclei [33, 77] and A14 nuclei [33] stimulate LH pul-

satite secretion. Oestradiol increases the extracellular concentrations of dopamine metabolites and the tyrosine hydroxylase activity of the dopaminergic A15 nucleus in ovariectomized ewes [24, 25]. Oestradiol also stimulates the nuclear expression of the *c-fos* protein in A14 and A15 nuclei during the long day period [48]. Dopamine from the A15 thus appears as an intermediate in the oestradiol negative feedback loop that affects LH secretion and decreases LH pulsatility during the long day period.

2.2. Sites of oestradiol action

The search for central sites of oestradiol action in female mammals, including ewes, mainly focused on positive feedback loops and sexual behaviour but the localizations where the steroid could act negatively during the oestrous cycle or seasonal anoestrus, remain poorly understood. In the ewe, the results of initial studies using intracranial oestradiol implants [18, 52, 71] have suggested that the mediobasal hypothalamus (MBH) and the AH play a role in oestrous induction. More recent studies on oestradiol implants in the VMH have shown that this structure is involved in the control of sexual behaviour [7] and the LH preovulatory surge [7, 12].

The absence of oestradiol receptors (E_2R) on LHRH neurons should be taken into account to better understand the mode of steroid action on pulsatile LH secretion. This has been shown in the rat [69], the guinea pig [90], the monkey [35] and the ewe [34, 45]. Steroids probably control the regulation of the pulsatile LH secretion using intermediate neuronal systems [21, 24, 25, 58, 77, 87]. However in the A15 nucleus, the effect of oestradiol on the dopaminergic metabolism probably results from direct activation. During the anoestrous season or constant long days,

Gallegos-Sánchez et al. [22] used an intracranial implantation technique to show that oestradiol acts directly on the lateral retrochiasmatic area (Rch), especially in the A15 nucleus, to control LH pulsatile secretion. This site of action thus differs from the one that stimulates sexual behaviour or the preovulatory LH surge [7]. Oestradiol implants in the VMH had no effect on LH pulsatility during the long day period in this experiment. It should also be mentioned that the localization of oestradiol activity for the inhibition of LH pulses during the long days in the lower anterior part of the hypothalamus in the ewe is similar to the localization of the inhibitory effects of oestradiol implants on LH pulsatile release observed in the dopaminergic nucleus from the periventricular anterior area of the hypothalamus in the female rat [3].

In the ewe, the neurons containing E_2R are mainly localized in the POA, the AH (figure 2), the ventrolateral septum, the bed nucleus of the stria terminalis, the VMH and the arcuate nucleus [8, 34, 47]. Lehman et al. [47] have also detected E_2R in the amygdala, the hippocampus and the periaqueductal grey matter. Oestradiol receptors are sometimes localized on dopaminergic or endorphine cells in the infundibular A12 nucleus [4, 45], on GABA cells in the preoptic area and on somatostatin cells in the VMH. More recently, distribution of E_2R and its possible photoperiodic change was investigated in female sheep under artificial light regimens [72]. There is a localization of E_2R in about 25 % of neurones from the A14 nucleus, which is independent of the photoperiod, and no E_2R in the A15 nucleus. These data could support the hypothesis of a cascade of events from the A14 to the A15 nucleus, and then to the LHRH system [33]. In contrast, photoperiodic changes in the distribution of E_2R involving a larger number of them during the long day period were observed in the

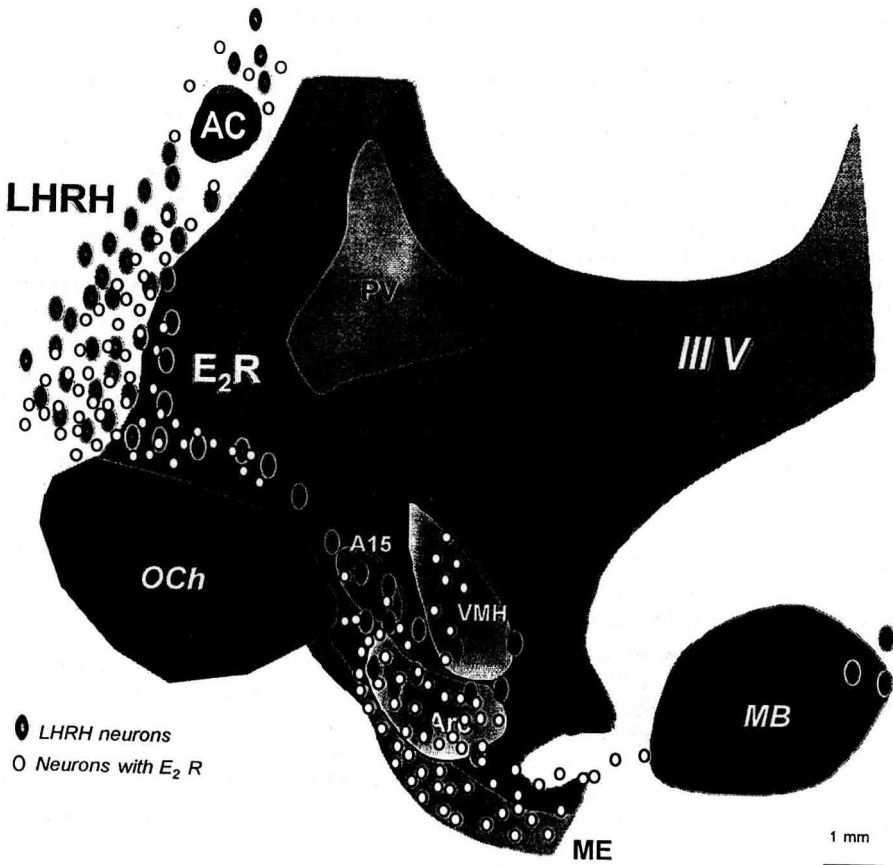


Figure 2. Schematic drawing of the hypothalamus of the sheep showing the distribution of LHRH neurons and the neurons bearing oestradiol receptors. OCh, optic chiasma; VMH, ventromedial nucleus; A15, dopaminergic A15 nucleus; PV, paraventricular nucleus; MB, mammillary bodies; Ar, arcuate nucleus; III/V, third ventricle. Redrawn from Blache and Martin [6].

preoptic area in a study by Skinner and Herbison [72]. The results from our work showing a direct effect of oestradiol in the A15 nucleus [22] either question the role of the receptors from the preoptic area and/or suggest the existence of several mechanisms involved in the regulation of LH release during the anoestrus.

It should be remembered that a neurochemical lesion of the A15 nucleus by 6-hydroxydopamine destroys the catecholaminergic elements, especially the

dopaminergic elements and provisionally increases the inhibition of LH pulsatility during the long day period [77]. A radiofrequency lesion has the same effect but lasts longer [33]. This difference between the effects of the neurochemical versus electrical lesions was interpreted as being due to the intensity of dopaminergic cell destruction. According to our recent results, the greater efficiency of the electrolytic lesions could also be considered as resulting from the destruction of

non-catecholaminergic elements, cell bodies and axon terminals having presently non-identified E₂R in A15.

2.3. Action of dopamine on the LHRH system

As in numerous species, the effects of dopamine on LHRH/LH secretion in ewe seem versatile [76]. In sheep, dopamine could participate in the inhibition of gonadotrophic activity before puberty in ewe lambs as well as in the adult ram during anoestrus. In both situations, pimozide and sulpiride, which are two dopaminergic antagonists, stimulate LH secretion [10, 85, 86]. Interestingly, Tortonese and Lincoln [85] propose a working hypothesis suggesting an interaction between dopaminergic and opioidergic inhibition and a change in the balance between both to help in the understanding of the seasonal regulation of LH secretion in sheep, but the mechanism remains to be established. In adult females, it has been shown that dopamine stimulates the amplitude of LH pulses in the pituitary gland during the breeding season [15] or just after puberty (Thiéry, unpublished data). It should also be mentioned that dopamine could act in the ventromedial hypothalamus of ewe to stimulate prolactin secretion through D1 receptors during the long day period [14]. Nonetheless, the role of dopamine in the regulation of LH secretion during seasonal anoestrus is the best documented. During the anoestrous season, the systemic injection of pimozide, a dopaminergic antagonist [57, 58], stimulates LH secretion. At least in adult ewes during the anoestrous season, the data suggest that dopamine acts on LHRH in the median eminence. For example, the dopamine concentration in tissues and the bioactivity of TH in the median eminence are higher during the long day period than during the short day period [75, 88]. Injection of pimozide [32] and alpha-methyl-

paratyrosine (α -MPT; [89]) in the median eminence stimulate pulsatile LH secretion in ovariectomized ewes treated with oestradiol. In the median eminence, the stimulation of D₂ receptors inhibits LH pulsatility [5]. The dopaminergic inhibition of LHRH cell secretion may consequently be of the presynaptic type and would occur in the terminals of neuron in the median eminence through the D₂ receptor [13]. However, the role of the A15 nucleus is not directly established for this inhibition in the median eminence since the terminals of this nucleus have only been found in the neurohypophysis [26]. In this structure the dopaminergic activity varies with that of the cell bodies as a function of photoperiodic or steroid conditions (Thiéry, unpublished data). Lesions in A15 lead to decreases in the dopamine contents of the median eminence [78] although its cells do not have any terminals in this structure. It is possible that a synaptic link between the A15 nucleus and the A12 nucleus via 'de passage' fibres exists [26, 48]. In the regulation of LH pulsatility, the A15 nucleus probably only gives a signal passing through the median eminence to amplify the inhibiting activity of the dopaminergic cells from the infundibular nucleus A12, which are, in turn, dependent on photoperiod.

3. SOME NEUROTRANSMITTERS INVOLVED IN THE REGULATION OF LH SECRETION IN THE EWES DURING ANOESTRUS

3.1. Serotonin

In the ewe, serotonin could play a role in the photoperiod inhibition of LH secretion [42, 43, 58]. In ovariectomized ewes supplemented with oestradiol, the supply of serotonergic receptor antagonists, i.e. cyproheptadine and ketanserine, under

various photoperiodic conditions combined with an inhibition of LH secretion leads to an increase in the number of LH pulses [42]. Moreover, similar to catecholamines, extracellular levels of serotonin, and particularly its metabolite 5-HIAA, are stimulated by oestradiol in the A15 dopaminergic nucleus [24]. Nevertheless, the stimulating effect of cyproheptadine on LH secretion is also observed in ovariectomized ewes non-supplemented with oestradiol. This suggests that serotonin also plays a role in the mechanisms involved in the photoperiodic regulation of LH secretion that are independent from those of oestradiol [58, 92].

3.2. Noradrenaline

In the ewe, noradrenaline could participate in the photoperiodic regulation of reproduction [15, 29, 57, 58, 64]. Meyer and Goodman [57, 58] obtained an increase in LH secretion by systemically injecting a noradrenergic antagonist, i.e. phenoxybenzamine, into intact ewes presenting seasonal anoestrus. Moreover, oestradiol increases the intra- and extracellular concentrations of a noradrenaline metabolite, 4-hydroxy-3-methoxyphenylethylene (MHPG) in the A15 nucleus during the long day period [24, 25, 75]. This suggests that noradrenaline may be involved in the establishment of negative feedback by oestradiol on LH secretion in this structure during anoestrus. It has been shown that there is intense innervation of the dopaminergic neurons of the A15 nucleus by noradrenergic terminals [83]. This innervation originates from noradrenergic cells of the mesencephalic A1 nucleus [84], a structure which presents E_2R in the rat [36]. However, the functional role of noradrenaline at this level is still not confirmed since blocking the noradrenergic activity in the A15 nucleus does not prevent either the stimulation of tyrosine hydroxylase (TH)

in these cells or the resulting inhibition of LH pulsatility [25].

3.3. GABA

As in rodents and primates, GABA could act on LHRH activity in the ewe, especially on its GABA_A receptor. During seasonal anoestrus, GABA stimulated the amplitude of LH pulses, involving GABA_B receptors [66, 67]. In the ovariectomized ewe, introducing a subcutaneous oestradiol implant during the long day period leads to an increase in the GABA concentration in the mediobasal hypothalamus 24 to 48 h later ([21]; *figure 3*). Moreover, it should be noted that extracellular GABA concentrations in the retrochiasmatic/A15 nucleus diminish between 48 h and 9 days later while the LH secretion is totally inhibited and dopaminergic activity is increased [79]. It should also be mentioned that glutamate, a stimulatory amino acid, follows the same time changes in this structure. It remains to be determined whether the opposing variations in levels of these two types of neurotransmitters represent only concomitant phenomena or whether there is a causal relationship.

4. CONCLUSION AND PERSPECTIVES

Up to now, of the various neurotransmitters which show modifications in the hypothalamus after oestradiol treatment, only dopamine seems to have a well-demonstrated involvement in the inhibition of LH pulsatile release during anoestrus in ewe. In the rat, inhibitory actions of dopamine have been shown in vitro as well as in vivo [19, 23]. Dopamine appears to be an intermediate in the negative feedback by oestradiol and testosterone on LH secretion [2, 16, 74]. It is interesting to note that dopaminergic inhi-

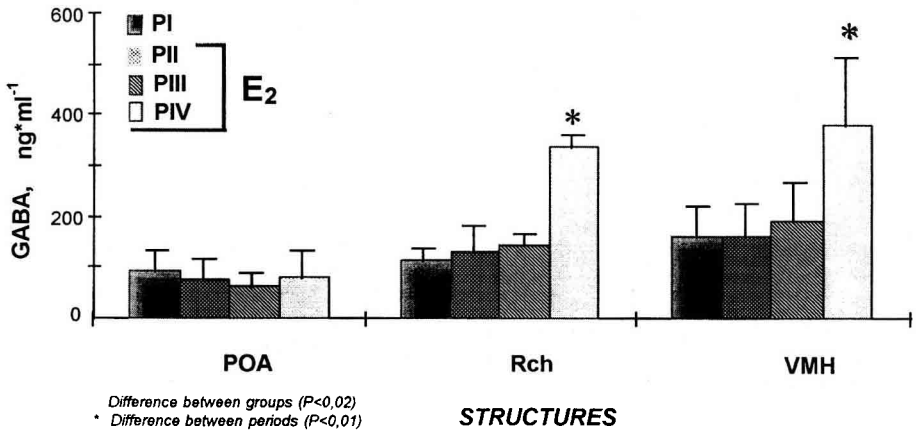


Figure 3. Changes in GABA contents of dialysates obtained in the preoptic area (POA), in the retrochiasmatic area (Rch), and in the ventromedial hypothalamus (VMH) of ovariectomized ewes. PI: before oestradiol infusion through the dialysis probe, PII from 0 to 4 h; PIII from 4 to 8 h and PIV from 20 to 24 h after the beginning of the oestradiol infusion. Note the increase in GABA within the Rch and VMH at PIV, when the frequency of LH pulses is beginning to decrease. Modified from Gallegos-Sánchez et al. [21].

bition of gonadotrophin secretion has been demonstrated for other groups of vertebrates (see Thiéry et al. [78] for a review). In fish, especially goldfish, dopamine acts directly on the gonadotrophs by inhibiting the effect of GnRH and also inhibits the secretion of the neuropeptide by a pre-synaptic inhibition through the D₂ receptors [62]. In rainbow trout, oestradiol receptors have been detected in the dopaminergic system inhibiting secretion of GTH2 gonadotrophin [49]. In *Rana temporata*, dopamine participates in gonadotrophic inhibition during hibernation [73] and, in birds, dopamine can also inhibit GnRH [68]. In these last two examples, a central effect is suggested for dopamine. The A15 nucleus in the ewe seems to maintain two levels of action, i.e. central and peripheral, but only the central level seems to act in seasonal regulations of gonadotrophic activity.

Current results showing a direct effect of oestradiol implants in the A15 nucleus,

where classical steroid receptors (now named E₂R α) have not been shown, raise questions about the local mode of action. According to the lack of effect of oestradiol implants on neighbouring structures such as the VMH, a diffusion of oestradiol from the implant in A15 to the E₂R of the close A14 seems unlikely. We need to determine the neuronal and glial cellular types, present in the lateral retrochiasmatic area, on which oestradiol acts. Oestradiol receptors different from those usually described might be involved. A new receptor, called receptor β , has been recently identified using molecular biological methods [40]. Using an in situ hybridization probe, Shughrue et al. [70] have recently shown the presence of mRNA for this receptor in the CNS. Some localizations are similar to those of receptor α , but some hypothalamic sites are different. The steroid may act on the release of a transmitter in a terminal innervating the A15 nucleus. Such phenomena have

already been described for other regulatory systems by Blaustein et al. [9]. Oestradiol membrane receptors, whose existence seems to be certain [60] can be located on nerve cell terminals. The direct action of oestradiol on the dopaminergic cells of A15 cannot be excluded. This phenomenon would be similar to that of the nigro-striatal system in the rat [61]. In this study, the phosphorylation of tyrosine hydroxylase is considered as a mode of activation of the enzyme. Phosphorylation is also contemplated as a mode of activation of the cells of the anteroventral periventricular nucleus in the rat [30]. In this nucleus, phosphorylation would concern the CREB protein and would occur before the activation of the cells, especially TH cells. Lagrange et al. [41] have recently shown that oestradiol effects were capable of modifying the electric activity of neurons deprived of receptors, more precisely the LHRH neurones of female guinea pigs. Electrophysiological methods using records of hypothalamic activity can thus be contemplated in the sheep.

Given the absence of terminals in the A15 nucleus of the median eminence and, particularly if the role of other neurotransmitters were to be demonstrated in the future, it could be hypothesized that the dopaminergic nucleus is not the effector or that it may not, in fact, play the inhibitory role that was proposed for it when it was initially identified. Rather, it may form part of an integrative structure participating in the regulation of LH pulsatility during anoestrus.

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