Evidence for dopamine D_1 receptor-mediated stimulation of prolactin secretion in ewes under long daylength

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Introduction

In seasonally breeding mammals, plasma concentrations of prolactin show marked seasonal variation, with concentrations high in summer and low in winter. This seasonal profile is caused by changing photoperiod: long daylengths stimulate prolactin secretion, while short daylengths have the opposite effect. In mammals, photic information is detected by the eye and transduced into an endocrine signal, that of nocturnal melatonin secretion, by the pineal gland (see Curlewis, 1992, for a review). Current evidence suggests that the melatonin signal is then detected in the mediobasal hypothalamus (MBH; Malpaux *et al.*, 1993; Lincoln, 1994) but the connection between this region and the lactotroph has yet to be characterized. If prolactin secretion in ewes is under the inhibitory control of dopamine, a long day melatonin signal would be expected to stimulate prolactin secretion by inhibiting the tuberoinfundibular dopaminergic neurones, thereby reducing dopamine concentrations in portal blood. However, research on several species has demonstrated that long daylengths activate, rather than suppress, dopaminergic pathways within the hypothalamus (Glass *et al.*, 1988; Thiéry, 1991). Furthermore, studies by Thomas *et al.* (1989a, b) suggest that dopamine may not be the prolactin inhibiting factor in sheep.

An increase in hypothalamic dopaminergic activity under long daylengths could be linked to prolactin secretion via effects on central dopamine D_1 receptors. We have shown that the D_1 receptor is positively coupled to prolactin secretion and that this effect is mediated via the hypothalamus and not directly at the pituitary (Curlewis *et al.*, 1994). In the present study, we hypothesized that an increase in dopaminergic activity under long daylengths could activate hypothalamic dopamine D_1 receptors that are positively linked with prolactin secretion. This hypothesis was tested by infusing a dopamine D_1 antagonist into two brain sites known to contain dopamine D_1 receptors (Curlewis *et al.*, 1992).

Materials and Methods

Ovariectomized Ile de France ewes were housed indoors under long daylength (16 h light:8 h dark) from 9 December 1992. On 22 February, they were shifted to short daylength (8 h light:16 h dark) and then returned to long daylength on 10 May. The drug infusion experiments occurred between 11 and 23 June, which was after 4–5 weeks of treatment with long daylength. About 3 months before the infusion experiment, bilateral guide tubes were surgically implanted, directed towards either the preoptic area (POA group; $n = 7$) or the ventromedial hypothalamic nucleus (VMH group; $n = 8$), with the tip of each guide tube 6 mm above the site of interest. Subcutaneous oestradiol implants (2 cm) were inserted approximately 2 weeks before the first infusion experiment. Each group was used in two trials. In the first trial, half of the ewes were infused with vehicle (0.9%, w/v, saline) and half with a D_1 antagonist (SCH23390; 2 nmol min^{-1}; 60 nmol total dose). To infuse into each brain site, a 26 gauge needle which protruded 6 mm beyond the tip of the guide tube was inserted and the test solution infused at 0.2 µl min^{-1} for 30 min. The pumps were then switched off and, after a further 5 min, the injectors were
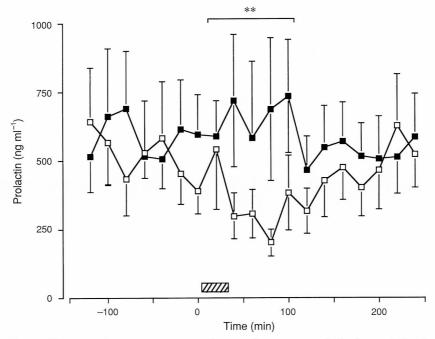
Fig. 1. Plasma prolactin concentrations from eight ewes in which the mediobasal hypothalamus was infused on separate occasions with vehicle (■) and the dopamine D_1 receptor antagonist SCH23390 (□; total dose 60 nmol l^{-1}). (▨): the period of infusion. The horizontal bar indicates the period when prolactin concentrations were significantly different between treatments. **$P < 0.01$, ANOVA.

removed. Blood was collected at 20 min intervals from 120 min before to 240 min after the start of the infusions. In the second trial one week later, the procedure was repeated with treatments reversed. Prolactin was measured by radioimmunoassay as described by Kann (1971). Data were divided into pre- and post-treatment periods and analysed by repeat measure ANOVA of log transformed data. Because there was a significant ($P < 0.05$) interaction between time and treatment for the post-treatment period of the VMH group, these data were further divided into two windows (20–100 min and 120–240 min), which were then analysed as described above. Placement of guide tubes by stereotaxis was verified by histology and with reference to a stereotaxic atlas of the sheep brain (Richard, 1969).

Results

During the sampling period, prolactin concentrations varied markedly with time and between animals. For the vehicle treatment, mean $\pm$ SEM prolactin concentration immediately before the start of infusions was 593 ± 146 and 513 ± 155 ng ml^{-1} for the VMH (Fig. 1) and POA (results not shown) groups, respectively. For each brain site, preinfusion prolactin concentrations were not significantly different between treatments (i.e. vehicle versus D_1 antagonist). For the VMH group, mean prolactin concentrations decreased during infusion of the D_1 antagonist to reach a nadir after 80 min. Thereafter, prolactin concentrations increased, returning to similar concentrations to those observed during infusion of the vehicle. For the period from 20 to 100 min after the start of the infusions, the suppressive effect of the D_1 antagonist was highly significant ($P < 0.01$). For the POA group, infusion of SCH23390 had no significant effect on prolactin concentrations. Placement of the guide tubes was verified by histology. The anterior–posterior position for the VMH group varied from A27.4 to A30.0 as defined in the stereotaxis atlas of Richard (1969). Three animals in this group did not show clear suppression of prolactin. In each of these animals, the anterior–posterior position was A30.0.
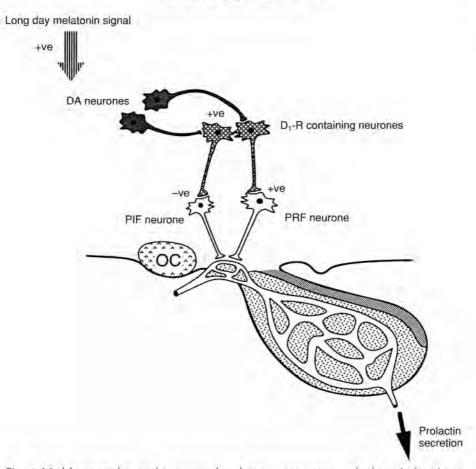
Fig. 2. Model proposed to explain increased prolactin concentrations under long daylengths. A long day melatonin signal stimulates dopamine (DA) containing neurones in the mediobasal hypothalamus. Dopamine D_1 receptors (D_1-R) are activated and stimulate secretion of a prolactin releasing factor (PRF) or inhibit secretion of a prolactin inhibiting factor (PIF) into the hypothalamo–hypophysial portal blood. OC· optic chiasma.

Discussion

This study provides the first direct evidence of dopaminergic stimulation of prolactin secretion mediated by hypothalamic dopamine D_1 receptors. Infusion of the D_1 antagonist into the VMH suppressed prolactin concentrations, while infusions anterior to the stereotaxis plane A30 had no effect. One explanation for this result is that increased dopaminergic activity in this area stimulates prolactin secretion and so antagonism of this effect in the present study resulted in suppression of prolactin. In further support of this idea, Thiéry (1991) has shown that long daylengths increase the tissue content of the dopamine metabolite, homovanillic acid, in the arcuate nucleus. Tissue samples from Thiéry's (1991) study were taken by a punch technique and would probably have included at least part of the region of interest in the present study. Nevertheless, further studies of dopaminergic activity are required to support the present hypothesis.

By incorporating recent observations on the site of action of melatonin with the findings discussed above, we propose a model (Fig. 2) as a framework for further research on the control of prolactin secretion by photoperiod. Melatonin microimplant studies suggest that the melatonin signal is detected and interpreted in the MBH. We propose that a long day melatonin signal stimulates one or other of the

dopamine cell groups which project to the VMH. Release of dopamine near D_1 receptors in this area activates a population of neurones which stimulate (directly or indirectly) secretion of a prolactin releasing factor or inhibit secretion of a prolactin release inhibiting factor.

A major challenge to this theory comes from a study by Lincoln and Clarke (1994), who show that surgical disconnection of the pituitary from the direct control of the hypothalamus does not abolish the influence of photoperiod on prolactin secretion. In addition, Lincoln (1994) showed that the MBH and, to a lesser extent, the pars tuberalis are sites where melatonin implants are effective in inhibiting prolactin secretion. In contrast, implants within the pars distalis are without effect on prolactin. Lincoln and Clarke (1994) argue that the most likely interpretation of these data is that the pars tuberalis is the target site for melatonin and that this area then interacts directly with the pars distalis to influence prolactin secretion. Furthermore, they suggest that the prolactin response to melatonin implants in the MBH is due to transport of melatonin from this region to the pars tuberalis by the rich vascular supply of this region. The hypothesis put forward by Lincoln and Clarke (1994) is in accord with an earlier study by Zinn *et al.* (1991) of bull calves. Zinn *et al.* (1991) show that dopamine turnover in the pituitary stalk and median eminence is not affected by daylength, despite large differences in plasma prolactin concentration. In contrast, research on mink has shown that the tyrosine hydroxylase content in the external zone of the median eminence is markedly reduced under long daylength (Boissin-Agasse *et al.*, 1991). Further evidence in support of hypothalamic involvement in photoperiodic control of prolactin secretion comes from studies in which hypothalamic lesions have been shown to modify the seasonal change in prolactin secretion (Thiéry *et al.*, 1979, 1989). The most simple explanation of the melatonin microimplant data is that the target site for this hormone is within the MBH and not the anterior pituitary (Malpaux *et al.*, 1993; Lincoln, 1994).

There are many unanswered questions in this field of research and differences due to species or experimental approach make interpretation of the small number of studies difficult. However, the major impediment to research in this area is that the identities of the physiologically important prolactin releasing factors and prolactin release inhibiting factors in sheep are not known. Further advances will be difficult until these have been identified.

Conclusion

In this study we have shown that infusion of a dopamine D_1 antagonist into the VMH inhibits prolactin secretion in ewes under long daylengths. These results are in accord with the hypothesis that prolactin secretion under long daylengths is stimulated by hypothalamic dopaminergic pathways that stimulate D_1 receptors located in the VMH. The pathways linking these receptors with prolactin secretion are yet to be determined.

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