Luteinizing hormone and prolactin are not retrograde transferred in perihypophyseal vascular complex in ewes

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SUMMARY

The objective of the study was to determine whether luteinizing hormone (LH) and prolactin (PRL) can access the brain by way of transfer from the venous blood of the cavernous sinus to the arterial blood supplying the brain and hypophysis. Studies were performed on heads of 22 mature sheep isolated during different phases of the estrous cycle and perfused with autologous blood. We were not able to demonstrate any transfer of LH and PRL in the investigated periods. This suggests that molecular weight of hormone may be a main factor determining the permeation and transfer of hormones in the perihypophyseal vascular complex. Reproductive Biology 2004 4 (2) : 195-201.

Key words: prolactin, LH, counter current transfer, ewes, breeding season, cavernous sinus

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INTRODUCTION

Previously, we demonstrated an existence of hormone transfer between vessels of the perihypophyseal vascular complex in female pigs and sheep. Radiolabelled substances of relatively small molecules, such as dopamine, steroids (progesterone, androstenol, testosterone) and neuropeptides (GnRH, β-endorphin, oxytocin) infused into the cavernous sinus were found in arterial blood supplying the hypophysis and brain [1, 2, 4, 5, 10-13, 15]. It was suggested that such system may be implicated in the regulation of some reproductive processes [2, 4, 13-15]. Grzegorzewski et al. [2] postulated that the transfer of GnRH in the perihypophyseal vascular complex may be included in the feedback loop regulation of GnRH secretion in pigs [2]. Evidence for short- or ultrashort-loop negative feedback for GnRH secretion in sheep has been presented by Padmanabhan et al. [6].

It is of interest that in the pig, protein hormones such as insulin (6 kDa) and relaxin (6.3 kDa) are transferred between vessels of the periovarian vascular complex [3, 7]. This may suggest that specific morphology of the perihypophyseal vascular complex creates conditions for transfer of molecules bigger than neuropeptides. To further understand the function of the perihypophyseal vascular complex in the regulation of reproduction, we examined whether protein hormones i.e. LH and prolactin (PRL) could be transferred from the venous blood of the cavernous sinus to the arterial blood supplying the brain and hypophysis.

MATERIALS AND METHODS

Adult, crossbred ewes (n=22) with controlled estrous cycle were assigned to one of the experimental group: the follicular phase (n=7), the early luteal phase (n=7) and the middle luteal phase (n=8) group. Experiments were performed on the model of perfused head isolated from animals after sacriﬁcation [9]. The isolated head was connected to the blood flow system by right common carotid artery. Blood samples for radioactivity measurement and hormones identification were collected from the catheter inserted into the left carotid artery. Ovine luteinizing hormone - (oLH; gift from Dr Y.
Combarnous, INRA, Nouzilly, France) and bovine prolactin (bPRL, USDA-bPRLL-I-2) were iodinated by the chloramine T method with 1mCi of Na$_2$-$^{125}$I (sp. act. 12.2 mCi/mg; Amersham International, UK). Total dose of 6.65 x 10$^7$ dpm of radiolabelled LH ($^{125}$I-LH) or radiolabelled prolactin ($^{125}$I-PRL) was dissolved in 10 ml of multielectrolytic liquid, and 5 ml of solution was infused for 5 min (1 ml/min) into each angularis oculi vein. Arterial effluent samples were collected at 1 min intervals from the left carotid artery beginning one minute prior to $^{125}$I-PRL and $^{125}$I-LH infusion (control samples for background estimation). The sampling was continued until 20 min after the beginning of labelled hormone infusion (experimental samples). Twelve ewes were used in the LH experiment (the follicular phase: n=4; early luteal phase: n=3; middle luteal phase: n=5) and 10 animals were used in the PRL experiment (the follicular phase: n=3; early luteal phase: n=4; middle luteal phase: n=3). The study was carried out in accordance with principles for the care and use of research animals and was approved by local Ethics Committee for Animal Experiments.

Control samples (1 ml) in ten replicates collected before PRL or LH infusion were measured for background estimation. Similarly, experimental samples (1 ml) in five replicates collected during and after the infusion were measured for 5 min with the LKB Wallac-Clinigamma (Rockville, MD) gamma counter.

To determine whether the radioactivity found in arterial blood represented unaltered $^{125}$I-LH the following experiment was carried out. The rabbit anti-oLH antiserum [9] (aLH/R-560; gift from Dr. B. Barcikowski, Jabłonna, Poland) was added to the samples of plasma taken from arterial blood collected from the carotid rete as well as samples containing a known level of radioactive LH. The concentration of antiserum was sufficient to bind 52% of radioactive LH. After incubation, anti-rabbit antibodies were added, samples were centrifuged, supernatant was removed and pellet counted with LKB Wallac-Clinigamma counter.

The mean background level of radioactivity estimated in 10 replicates collected before labeled hormone infusion was subtracted from each measurement of experimental sample collected in the same experiment. Statistical differences between radioactivity level in control
samples and radioactivity measured in samples collected during and after labeled hormone infusion were estimated by T test (GraphPad, San Diego, CA, USA). The area under the curve (AUC) for total radioactivity measured during the first 10 minutes of blood sampling was calculated (GraphPad) for each experiment. Then the mean (±SEM) AUC was calculated for the follicular, early and middle luteal phase. The differences between phases of the estrous cycle were analyzed by one-way analysis of variance after log transformation and followed by a Bonferroni test (GraphPad).

RESULTS AND DISCUSSION

Infusion of $^{125}$I-LH into the cavernous sinus resulted in a significant (p<0.05) increase in radioactivity above background in arterial blood collected from the carotid rete in all investigated periods: the follicular, early luteal and middle luteal phases of the ovine estrous cycle (fig. 1A). The highest (p<0.05) mean radioactivity was found in blood samples collected during the middle luteal phase (fig. 1B).

The experiments with the rabbit anti-oLH antisera, however, demonstrated that radioactivity found in blood taken from the carotid artery did not represent immunoreactive LH. This radioactivity represented probably some unidentified smaller fragments of LH. There are two possible explanations for the presence of radioactivity in arterial blood: instability of labelled LH infused into the cavernous sinus or the degradation of $^{125}$I-LH after infusion. It is known that LH after binding to its receptor is internalized and degraded within lysosomes [8]. In our previous work we have demonstrated the presence of LH/hCG receptor mRNA and proteins in walls of both arterial and venous compartments of the cavernous sinus - carotid rete complex in sheep [9]. Therefore, the presence of radioactivity in arterial blood may result from the transfer of fragments of $^{125}$I-LH after hormone degradation in the tissues rather than infusion of unstable $^{125}$I-LH.

Infusion of $^{125}$I-PRL into the cavernous sinus caused no increase in radioactivity in arterial blood collected from the carotid rete. The described
here lack of LH (30 kDa) and PRL (23 kDa) transfer from the venous blood of the cavernous sinus to the arterial blood of the carotid rete together with earlier findings concerning transfer of dopamine (0.19 kDa), testosterone (0.29 kDa), progesterone (0.32 kDa), oxytocin (1.0 kDa), GnRH (1.2 kDa) and β-endorphin (3.4 kDa; [1, 2, 10-14]) suggest that molecular weight of hormone may be a main factor determining the permeation and transfer of hormones in the perihypophyseal vascular complex. Regardless of the nature of the substance found in arterial blood collected during the infusion of $^{125}$I-LH, higher mean radioactivity (p<0.05) measured in the middle luteal phase compared with other investigated periods seems to confirm our previous data demonstrating an influence of the estrous cycle on the transfer of hormones in the perihypophyseal vascular complex [1, 2, 10].

In conclusion, the present data demonstrated that in the sheep, LH and PRL were not retrograde transferred from the venous blood of the cavernous sinus into the arterial blood supplying the brain and hypophysis. Therefore, in the sheep the protein hormones are not able to reach the brain structures through the local vascular pathway.

*Fig. 1.* A. Radioactivity values (mean±SEM) in arterial blood samples collected after $^{125}$I-LH infusion into the cavernous sinus of sheep. A significant (p<0.05) increase in radioactivity was found in the follicular phase (dotted line; n=4), early luteal phase (solid line; n=3) and middle luteal phase (dashed line; n=5). B. Total radioactivity calculated for the first 10 minutes of blood sampling from the carotid rete in each phase represented as the area under the curve (relative units, mean±SEM). Bars with different superscripts were significantly different (p<0.05). F - follicular phase, EL - early luteal phase, ML - middle luteal phase.
REFERENCES


