OPIOID INHIBITION OF THE PULSATILE LUTEINIZING HORMONE RELEASE AS ASSESSED BY NALOXONE TREATMENT IN THE LACTATING RABBIT

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ABSTRACT

Since in nursing rabbits sexual receptivity and fertility achieved after AI appear to be depressed during lactation, the effect of endogenous opioid peptides (EOP) on serum LH were investigated in lactating rabbits (n=30) by administration of the opiate antagonist naloxone (nal). Blood samples were collected every 15 min for 4 h via an indwelling catheter inserted in the central ear artery. After the first h of sampling, the rabbits received i.v. saline (SAL, n=10); nal (LN, 0.5 mg/kg, n=10); or nal (HN, 1.0 mg/kg, n=10). The pulsar algorithmic procedure for the study of pulsatile hormone secretion was used to calculate mean and basal LH concentrations, frequency and amplitude of LH pulses. Data from the statistical analysis are presented as mean ± SE of area under the curve (AUC) units of LH response. Mean LH concentration during the 60 min pre-challenge period was 0.24±0.03 ng/ml. The AUC of LH during that period did not differ among treatment groups (LN=16.6±4.1, HN=11.8±2.9 and SAL=14.3±4.0 area units). In the SAL group LH secretion remained low during the 4 h sampling period. Mean and basal LH concentrations were 0.21±0.05 ng/ml and 0.03±0.02 ng/ml (LN=0.59±0.13 ng/ml and 0.20±0.04 ng/ml; HN=0.38±0.09 ng/ml and 0.13±0.03 ng/ml). The mean pulse peak was 0.70±0.15 ng/ml (LN=1.95±0.37 ng/ml; HN=1.26±0.23 ng/ml), and the mean pulse amplitude was 0.67±0.14 ng/ml (LN=1.89±0.36 ng/ml; HN=1.22±0.21 ng/ml). A lower number of pulses (1.88±0.35) was also detected for the 4 h period (LN=2.57±1.13; HN=2.34±1.11). Nal treatment increased LH release. A greater AUC was observed during the 60 min post nal period in both nal-treated groups (LN=55.0±15.5 and HN=38.8±10.3 vs. SAL=5.9±2.7 area units; $P<0.01$). Rabbits receiving 0.5 mg/kg nal had an increased ($P<0.05$) AUC (LN=105.9±26.1 area units) through 180 min after nal administration compared with the saline-treated group (SAL=34.9±9.2 area units). The group LN differed from the SAL group through the 180 min post-nal period, while the HN group differed only for 60 min. Nal-treated groups (LN and HN) did not differ in either of the post-nal periods (60 and 180 min). Since all the experimental rabbits presented a clear LH surge after the nal challenge, the suppression of pituitary LH release has been shown to be associated with EOP activity. EOP may have modulated hypothalamic secretion of GnRH, resulting in a tonic inhibition of LH secretion. However, further studies are necessary to better clarify EOP-cortisol interaction in the lactating rabbit doe at the moment of AI and after an i.m. injection of a GnRH analogue to induce ovulation.

Key words: Rabbit, Lactation, Endogenous opioid peptides, Luteinizing hormone.

INTRODUCTION

Research of factors responsible for successful artificial insemination (AI) has been the object of numerous studies in the recent years. Even though rabbit does can be inseminated just after kindling, their reproduction efficiency varies considerably with parity order, physiological state (lactating or not, stage of lactation) and sexual receptivity at insemination (reviewed by Theau-Clément, 2007). In nursing rabbits, as in other species, sexual receptivity and fertility achieved after AI appear to be depressed during the period of lactation. The existence of a partial antagonism between lactation and reproduction, reflecting the corresponding hormonal antagonism between PRL and release of gonadotropins, has been widely reported (Theau-Clément and Roustan, 1992; Forthun Lamothe and Bolet, 1995; Boiti, 2004). While the effects of PRL on the level of the uterus and ovary seem rather

well known, the mechanisms of action of PRL on the level of the hypothalamo-pituary axis, are not clearly identified. The secretions of dopamine and endogenous opioid peptides (EOP) at the hypothalamic level, intervening in the release of PRL, would be implicated in the decreased secretion of gonadotropins (reviewed by Theau-Clément, 2007).

As a matter of fact, EOP have been indicated as modifiers of gonadotropin release in several species, including humans. In particular, McNeilly (1988) and Marongiu (1995) suggested that the inhibition of LH during lactation might be a result of an increased secretion of β-endorphin produced by suckling in ewes. However, the opioidergic involvement in the lactating rabbit has not been characterised as precisely as in other domestic species. Therefore, the present experiment was conducted to determine EOP interaction in the suckling-induced suppression of LH release in the rabbit by administration of the opiate antagonist naloxone.

MATERIALS AND METHODS

Animals and experimental design

Thirty multiparous hybrid rabbit does during lactation were utilized to carry out the present study. The animals were kept isolated from one another, being caged individually, under controlled conditions of light (14L:10D schedule) and with food and water available *ad libitum*. Experimental rabbits were randomly allotted into three groups receiving three different treatments.

Blood sampling

Blood sampling was performed via an indwelling Teflon catheter, 3.2 cm long, 20 gauge which had been inserted in the central ear artery. The blood samples were obtained every 15 min for 4 h. After the first hour of sampling, the rabbits received the following intravenous (marginal ear vein) treatments: low naloxone (LN, 0.5 mg/kg naloxone diluted in saline, n=10); high naloxone (HN, 1.0 mg/kg naloxone, n=10); or saline (SAL, control, n=10). Blood samples were processed to yield serum and were stored at -20ºC until assayed for LH by radioimmunoassay (RIA). The protocol involving the care and use of the animals for this experiment was approved by the Bioethic Committee of the University of Sassari. Moreover, the study was performed according to the CEE Council Directives (86/609, 1986) for the care of experimental animals.

LH assay

Serum LH concentrations were determined in each serum sample by means of a specific homologous double antibody RIA technique. Duplicates (200 µl) of each sample were taken and diluted with 300 ml of 1.0% BSA-PBS. The first antibody was then added in 0.1 ml of PBS containing 1:200 normal guinea pig serum. This mixture was incubated for 24 h. At the end of this 24 h incubation, the ^{125}labeled LH was added in 0.1 ml PBS. After a second 24 h incubation, the anti-guinea pig gamma globulin was added in 0.2 ml PBS and the incubation continued for an additional 2 days. The hormone antibody complex was collected by centrifugation, the supernatant fractions discarded, and the ^{125}I content of the precipitates determined in a Beckman gamma counter. The sensitivity of the assay was 0.04 ng. The intra-assay coefficient of variation (CV) was 18.1%.

Data analysis

The pulsar algorithmic procedure for the study of pulsatile hormone secretion (Merriam and Watcher, 1982) was used to calculate mean and basal LH concentrations, frequency and amplitude of LH pulses in individual profiles of the serial sampling period. The following definitions were used for this analysis: mean concentration (average of all LH concentrations); trough point (LH concentrations at one standard deviation below the mean LH concentration); basal concentration (average of all the trough points); pulse (any elevation in LH concentrations greater than one standard deviation above

the mean LH concentration); pulse height (highest increase within an LH pulse); pulse amplitude (difference between LH pulse height and basal LH concentration); pulse frequency (number of pulses every 4 h). The LH patterns were analysed by determining the area under the curve (AUC) of hormone release which represented the pituitary LH total response to treatments over the test period. Serum LH concentrations were transformed into AUC of response as the area of a polygon by the trapezoidal formula. These data were subjected to analysis of variance using the SAS (1998) GLM procedure and differences among treatments were determined by the least square means method. Results from the statistical analysis are presented as mean ± SE area under the curve (AUC) units of LH response and a $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Serum LH profile over the test period, as assessed by measuring mean LH concentrations of the three treatment groups, is depicted in Figure 1.

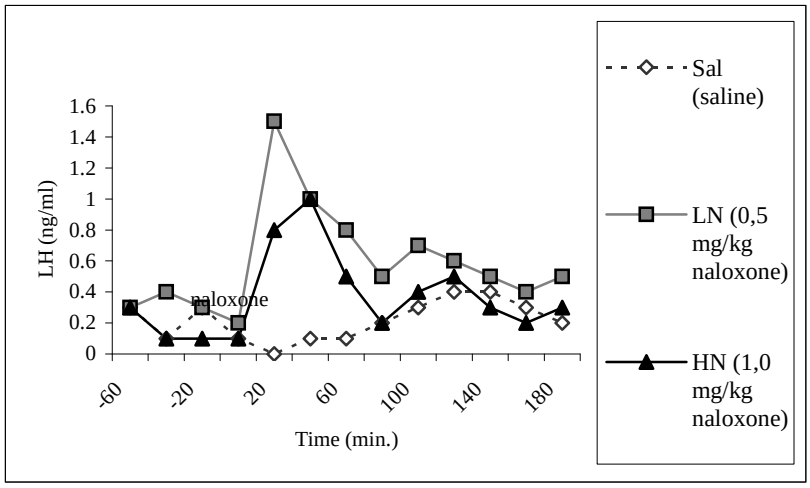

Figure 1: Mean serum LH concentrations one hour before and three hours after naloxone or saline treatment

When serum LH parameters were analysed during the 4 h of the sampling period in the SAL group, LH secretion remained low. Mean and basal serum LH concentrations were 0.21 ± 0.05 ng/ml and 0.03 ± 0.02 ng/ml (LN = 0.59 ± 0.13 ng/ml and 0.20 ± 0.04 ng/ml; HN = 0.38 ± 0.09 ng/ml and 0.13 ± 0.03 ng/ml). The mean pulse peak was 0.70 ± 0.15 ng/ml (LN = 1.95 ± 0.37 ng/ml; HN = 1.26 ± 0.23 ng/ml), and the mean pulse amplitude was 0.67 ± 0.14 ng/ml (LN = 1.89 ± 0.36 ng/ml; HN = 1.22 ± 0.21 ng/ml). A lower number of pulses (1.88 ± 0.35) was also detected for the 4-h period (LN = 2.57 ± 1.13; HN = 2.34 ± 1.11). Results from the statistical analysis are expressed by presenting the area under the curve (AUC) of LH response in Figure 2.

Mean serum LH concentration of the experimental rabbits during the 60-min pre challenge period was 0.24 ± 0.03 ng/ml. AUC during that period did not differ ($P>0.6$) among treatment groups (LN = 16.4 ± 4.1, HN = 11.6 ± 2.9 and SA= 14.1 ± 4.0 area units. Naloxone treatment increased LH release. A greater AUC was observed during the 60 min post naloxone period in both naloxone-treated groups when compared with the saline-treated group (LN = 56.8 ± 15.5 and HN = 40.6 ± 10.3 vs. SAL = 4.5 ± 2.7 area units; $P<0.01$). Rabbits receiving 0.5 mg/kg naloxone, had an increased ($P<0.05$) AUC (LN = 105.9 ± 26.1 area units) through 180 min after naloxone administration compared with the saline-treated group (SAL = 40.0 ± 9.2 area units).

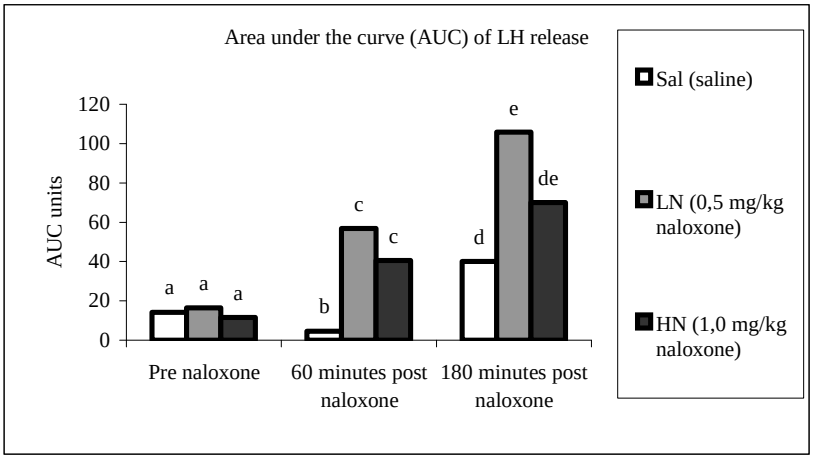

Figure 2: AUC of LH response at pre treatment and at 60 and 180 minutes post naloxone treatment. Each bar represents the mean area units of ten values. Bars with different superscripts within the same time point over the test period differ at least $P<0.05$

The group LN differed from the SAL group through the 180-min post naloxone period, while the HN group differed only for the 60-min post naloxone period from the SAL group. Naloxone treated groups (LN and HN) did not statistically differ in either of the post naloxone periods (60 and 180 min). A similar pattern has been reported in postpartum anestrous beef cows (Whisnant *et al.*, 1986a), confirming that increasing dosages of naloxone, if the critical threshold to counteract EOP inhibitory effects have already been exceeded, do not produce a graded LH response during lactation.

In the present trial, naloxone increased serum LH concentrations in lactating rabbit does. Since all the experimental rabbits responded to the treatment by presenting a clear LH surge after the naloxone challenge, the inhibition of pituitary LH release has been shown to be associated with EOP activity. As proposed by Kalra and Kalra (1984), EOP may have modulated hypothalamic GnRH secretion indirectly by suppressing hypothalamic adrenergic activity, resulting in a tonic inhibition of LH secretion.

Our results appear in agreement with previous studies postulating that EOP may modulate LH secretion in different species. Suckling elicits an increase in serum concentrations of β-endorphin concentrations in the lactating rat (Riskind *et al.*,1984) and the suckling-induced suppression in serum LH concentrations can be blocked by administration of EOP-receptor antagonist naloxone (Sirinathsinghji and Martini, 1984). Furthermore, EOP may inhibit LH secretion during the postpartum period in domestic species. Whisnant *et al.* (1986a) and Newton *et al.* (1988) reported that naloxone increased serum LH concentrations in postpartum anestrous beef cows and ewes, respectively. Barb *et al.* (1986) observed a serum LH surge due to naloxone in lactating sows.
Moreover, our observation that EOP act as mediators of the suckling-induced depression of LH re1ease in the rabbit could support previous findings regarding the doe-litter separation method (Ubilla *et al.*, 2000). As a matter of fact, this particular technique of biostimulation, being associated with the absence of suckling episodes and thus low EOP secretion, would lead to a higher LH response and, therefore, exert a major effect on fertility.

However, some additional factors need to be taken into account when the influences of EOP on LH release are investigated. In particular, stress occurring in response to the handling of rabbit does, peculiarly during AI procedures, can produce increased cortisol re1ease which inhibits LH secretion. Indeed, high cortisol concentrations have been reported to depress basal and GnRH-induced LH release in several domestic species (Echternkamp, 1984). Thus, further studies are necessary to better clarify EOP-cortisol interaction in the lactating rabbit doe at the moment of AI and after an intramuscular injection of a GnRH analogue to induce ovulation.

CONCLUSIONS

In conclusion, the results from the present investigation indicate that the opiate antagonist naloxone clearly elevated serum LH in suckled rabbit does, suggesting an EOP role in the inhibition of LH secretion during the lactation period in this species. Nevertheless further research is needed to clarify EOP interactions with other main factors involved in the antagonism between lactation and reproduction.

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