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Original article

Reduction of ovarian oxytocin content from early luteal phase does not affect the corpus luteum secretory function in cattle

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Summary — It was found previously that the reduction of ovarian oxytocin (OT) content by up to 80% on d 12 and 16 of the cycle affects neither luteolysis nor estrous cycle duration in cattle. Because ovarian OT is suggested to be involved in progesterone synthesis/secretion, in the present study we wanted to investigate whether ovary depletion of OT in the early stages of the estrous cycle will influence the corpus luteum (CL) secretory function. In experiment 1, heifers (n = 15) had cannulae inserted into the aorta abdominalis through the coccygeal artery. The tip of each cannula was placed cranial to the origin of the ovarian artery. Noradrenaline (NA; 4 mg) was infused on d 5–10 for 30 min daily, evoking release of OT and lower, but evident, increases in the progesterone levels. This did not affect the length of the estrous cycle compared with control heifers infused with saline only. Because ovarian OT cannot be restored once discharged, NA served as a tool to deplete the CL of OT in experiment 2. The same dose of NA was given on d 5–10 to 4 heifers. The corpora lutea used to determine OT tissue concentrations were obtained by surgery under local anaesthesia 10–15 min after the last NA infusion. This dose of NA, depleted CL of OT by about 51% compared with control heifers. We conclude that significant reduction of ovarian OT content on d 5–10 does not affect the CL function. Thus, if ovarian OT is involved in ovarian steroidogeneis, it plays a modulatory rather than a mandatory role.

corpus luteum / oxytocin / noradrenaline / estrous cycle / cattle

Résumé — La réduction de la concentration de l'ocytocine ovarienne au début de la phase lutéale n'influe pas sur la fonction du corps jaune chez la vache. *Des recherches antérieures ont démontré que la diminution du taux d'oxytocine (OT) de 80% entre le 12e et le 16e jour du cycle œstral n'influe pas sur la durée du cycle chez la vache. Pour tester un rôle possible de l'OT ovarienne sur la synthèse/secrétion de la progestérone, nous avons vérifié si la déplétion de l'OT contenue dans l'ovaire au début de la phase lutéale influençait la fonction sécrétoire du corps jaune (CL). Chez les génisses (n = 15) des canules ont été introduites dans l'artère abdominale à partir de l'artère caudale de façon à ce que les terminaisons de chaque canule soient placées frontalement à l'origine de l'artère ovarienne. L'infusion de noradrénaline (NA; 4 mg) pendant 30*

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mn du 5e au 10e jour provoque une augmentation de la secrétion d'OT, et une faible augmentation du niveau de progestérone. Cela n'influe pas sur la durée du cycle comparativement aux génisses du groupe témoin auxquelles a été infusé du liquide physiologique. Puisque l'oxytocine, une fois sécrétée, ne peut plus être resynthétisée, la NA est utilisée comme moyen de déplétion du CL de l'OT dans l'expérience 2. La même dose de NA a été administrée du 5e au 10e jour chez 4 génisses. Dans le but de déterminer la concentration de l'OT du tissu après une anesthésie locale, le CL a été prélevé chirugicalement 10 à 15 mn après la dernière infusion de NA. Cette dose de NA, entraîne une déplétion du CL d'environ 51% d'OT comparativement au groupe témoin. Nous concluons que la nette réduction de la concentration de l'OT ovarienne durant la période du 5e au 10e jour n'influe pas sur la fonction lutéale. Donc si l'OT ovarienne influe sur la stéroïdogenèse lutéale, son rôle est plutôt modulateur que principal.

corps jaune / oxytocine / noradrénaline / cycle œstral / vache

INTRODUCTION

Oxytocin (OT) synthesis in the bovine corpus luteum (CL) works in exactly the same way as in hypothalamic nuclei (Swann *et al,* 1984). The first step is biosynthesis in rough endoplasmic reticulum of a prohormone consisting of OT coupled with neurophysin and signal peptide (Schmale and Richter, 1980). The synthesis of prohormone lasts as long as OT-mRNA is available, *ie* until d 5–7 of the estrous cycle (Fehr *et al,* 1987). The highest concentration of OT-mRNA was shown on d 2–4 after ovulation (Ivell and Richter, 1984). On the other hand, the highest concentration of OT in cattle (447 ng/g CL) was found on d 8–12 of the cycle, whereas around luteolysis the amount of OT in the CL was dramatically decreased (74 ng/g) (Schams *et al,* 1985). Studies of cattle *in vitro* (Luck and Jungclas, 1987) and *in vivo* (Kotwica *et al,* 1991b) have shown that catecholamines stimulate ovarian function. The stimulatory effect of noradrenaline (NA) upon ovarian OT secretion has an efficiency comparable to that of $PGF_{2\alpha}$ (Kotwica *et al,* 1991b) which releases about 98% of the hormone stored in the CL (Flint and Sheldrick, 1982). Since ovarian OT cannot be replaced once released (Ivell *et al,* 1985), NA can be used to deplete the bovine CL of OT to determine its physiological function. Reduction of ovarian OT content by 65–82% using NA on d 12 and 16 did not affect luteolysis or the duration of the estrous cycle (Kotwica and Skarzynski, 1993). Hence we assumed that ovarian OT may have a permissive, rather than a direct, action on luteolysis in cattle. If so, then an alternative role of ovarian OT could be its regulatory function in the early and middle CL. This speculation is supported by Miyamoto and Schams (1991), who stimulated progesterone release by OT at the early luteal cells in the microdialysis system. Moreover, the highest specific binding of OT on bovine luteal cells was found on d 8–12 of the cycle (Okuda *et al,* 1992). There-fore, in our studies we infused NA on d 5–10 of the cycle, *ie* just after OT prohormone synthesis was finished (Fehr *et al,* 1987) but at the time when OT was suspected to be involved in the CL function, to examine how it affects the length of the estrous cycle.

MATERIALS AND METHODS

Animals and surgery

Mature heifers (380–450 kg) with regular ovarian cyclicity (18–22 d) were used in this study. The day of onset of heat was accepted as day zero of the estrous cycle. One day before the study, a PVC catheter (OD 1.7 mm, ID 1.2 mm) was inserted into the abdominal aorta through the coccygeal artery (Kotwica *et al,* 1990) to facilate infu-

sion of either saline or NA. The tip of each cannula was positioned cranial to the origin of the ovarian artery to allow direct application of drugs to the reproductive tract. Such local delivery permitted the use a lower dose of drug, hence avoiding side effects. The control of the catheter placement in aorta abdominalis has been described previously (Kotwica *et al,* 1990). Another catheter was inserted into the jugular vein to collect blood samples.

Corpora lutea collection

To collect the CL, animals were tranquillized with 0.06 mg/kg body weight of xylazine (Rometar, Spofa) and then anesthetised locally with 10–15 ml 3% Polocaine (Polocainum hydrochloricum, Biovet) given paracranially at 3 points between the first and fourth lumbar. Ovaries, or only the CL is possible, were obtained by means of an effeminator (Hauptner, FRG), by colpotomy, or by lumbar incision. These were placed in liquid nitrogen. To reduce the stress effect, all surgery was performed in the barn where the animals were standing.

Experiment 1

To study the effect of NA on CL secretion in the early stages of the cycle, 4 mg NA (Levonor, Polfa) in 10 ml saline was infused into the aorta abdominalis in 4 heifers for 30 min daily on d 5–10 of the estrous cycle. Saline (10 ml) was given in the same way during a control period lasting 1 h before and after drug infusion. Blood samples were taken every 5 or 10 min during NA and saline infusion, respectively. Moreover, blood samples were taken once daily from d 0–4 and 11–23 of the cycle to confirm its duration. Three other heifers treated as above but with saline, were bled once daily during the cycle and served as a control. Blood samples (8 ml) were collected in tubes containing 30 μM EDTA and 1% acetylsalicylic acid (Meyer *et al,* 1989), chilled in an ice-bath, and then centrifuged at 4°C. Plasma was stored at –20°C.

Experiment 2, preliminary experiment

To adjust the method of OT determination in luteal tissue (Tsang *et al,* 1990), the CL samples from heifers were collected in the slaughterhouse and stored in liquid nitrogen. The ages of the CL samples were estimated on the base of size, color, consistency and presence of superficial blood vessels (Ireland *et al,* 1980). OT was studied in CL from the 3 stages of the cycle: d 4–7; 8–13; and 16–20 (Schams *et al,* 1985).

The aim of experiment 2 was to determine OT content in the luteal tissue from heifers ($n = 4$) infused with NA as in experiment 1 on d 5–10 of the cycle. On d 10, 10–15 min after the last NA infusion was stopped, CL was obtained surgically. The CL obtained on d 10 from 4 heifers infused with saline from d 5–10 served as controls.

Hormone determinations

Progesterone concentration was determined by RIA (Kotwica *et al,* 1990) using ovine progesterone antiserum (GDN No 337, donated by GD Niswender) and characterized by Gibori *et al* (1977). The sensitivity of the procedure was 15 pg/tube, *ie* 0.3 ng/ml of plasma. The intra- and interassay variations were 8.1 and 15.9%, respectively. The relationship between real and determined amounts of 4 different concentrations of progesterone added to the plasma samples is expressed by the linear regression equation $y = 1.034x - 0.13$.

OT plasma levels were determined by using ice-chilled acetone for extraction (Schams *et al,* 1979). Rabbit OT antiserum (R–1, donated by G Kotwica) was characterized previously (Kotwica and Skarzynski, 1993). The efficiency of extraction was 85% and final data were corrected for procedural losses. The sensitivity of the method was 3 pg/ml. The intra- and interassay variations were 7.5 and 14.6%, respectively. The precision of the procedure was expressed by the linear regression equation ($y = 0.99x + 0.14$).

Plasma PGFM concentrations in blood samples from heifers in experiment 1 and determined by radioimmunoassay as described by Homanics and Silvia (1988). The antiserum (WS4468 BD 6/23-7/21; donated by WJ Silvia) and free and antibody-bound tracers were separated by charcoal precipitation. The sensitivity of the assay was 50 pg/ml, and the intra- and interassay coefficients of variation were 5.6 and 9.0%, respectively.

Data analysis

Data from experiments 1 and 2, including the infusions of NA on d 5–10, were combined for further analysis. The total amount of OT and progesterone secreted into the blood during NA infusion was calculated by measuring separately the area under the curve for each animal. Mean concentrations of each hormone at the pre-infusion period were accepted as the zero point. Differences between mean values (± SEM) were estimated by one-way analysis of variance.

RESULTS

A few minutes after the start of NA infusion in all animals of experiments 1 and 2, a significant increase ($P < 0.01$) in OT and a smaller but evident ($P < 0.05$) increase in progesterone were found in the peripheral blood (fig 1). The amount of OT secreted by NA depended upon the day of the estrous cycle, continuously increasing ($P < 0.05$) from d 5–8 (fig 2). Secretion of progesterone stimulated by NA also rose ($P < 0.05$) from d 5–7 of the estrous cycle (fig 2). Time of luteolysis, measured by progesterone levels falling below 2 ng/ml (Roche *et al*, 1981) in heifers treated with NA (18.3 ± 0.4 d), was similar (fig 3) to the control (18.6 ± 0.3).

In preliminary studies of experiment 2, the highest ($P < 0.001$) concentrations of OT in the slaughterhouse CL were shown in the middle (438 ± 53 ng/g), compared with the early (23.5 ± 4.4 ng/g) and late (14.7 ± 8.4 ng/g) luteal phases. The mean OT concentration in the CL after NA infusion on d 10 was 184 ± 28.9 ng/g tissue and it was more than 50% less ($P < 0.001$) than in the control group (360.8 ± 37.4 ng/g) (table I). In 1 of 4 NA-infused heifers, the CL content of OT was slightly lower (289 ± 54.8 ng/g) compared with the control. However, in this heifer, a markedly lower amount of OT secretion was shown during NA infusion, supposedly due to incorrect placement

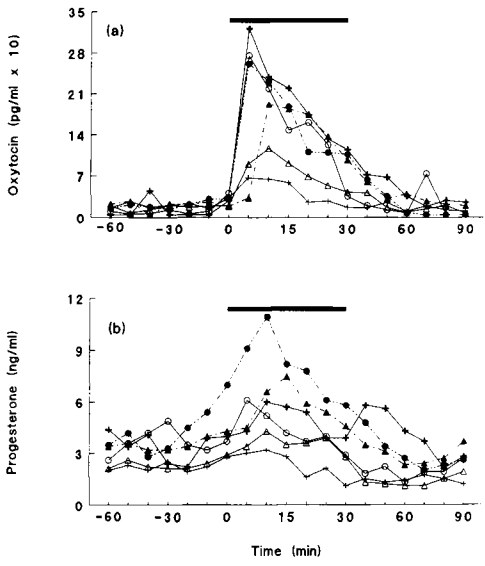

Fig 1. Oxytocin (a) and progesterone (b) plasma concentrations increase during noradrenaline (4 mg/30 min) infusion (horizontal bar) into the aorta abdominalis on d 5 (–I–), 6 (–Δ–), 7 (–O–), 8 (–+–), 9 (–▲–) and 10 (–●–) in one representative heifer (No 06437) before and after noradrenaline infusion saline was given.

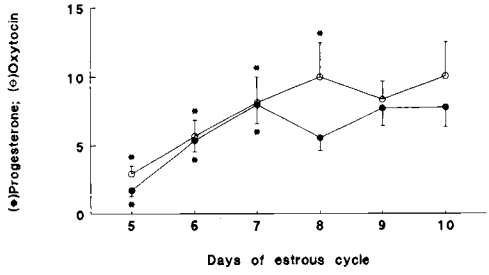

Fig 2. Mean (± SEM) increase of oxytocin and progesterone plasma concentrations (area under the curve; relative units) during noradrenaline infusion (4 mg/30 min) on d 5–10 ($n = 8$). Mean concentrations of hormones, before infusion of noradrenaline on each day, accepted as zero point. * Increased linearly from 5–7 for progesterone and 5–8 for oxytocin ($P < 0.05$).

of the tip of catheter in the aorta abdominalis. Therefore, this result was excluded from the mean value of the group (table I). PGFM concentrations were below the sensitivity of method (50 pg/ml) in all studied plasma samples (data not given).

DISCUSSION

NA infusions on d 5–10 of the estrous cycle (experiments 1 and 2) increased OT secretion and caused a significant, but less evident, rise of progesterone in all the studied heifers. It should be emphasized that, although NA affects neural OT secretion (Parker and Crowley, 1993), if given to the aorta abdominalis it predominantly stimulates the release of ovarian OT (Kotwica *et al,* 1991b). Stimulation of CL steroidogenesis in cattle by the adrenergic system occurs through β-adrenoceptor activation (Skarzynski and Kotwica, 1993), and blocking these receptors with propranolol, decreased progesterone secretion *in vitro* (Godkin *et al,* 1977) and *in vivo* (Kotwica *et al,* 1991a). β-Receptors in the CL of cattle depend on the stage of the cycle and their concentration was the highest on d 4 as compared with any other studied day of the cycle (Pesta *et al,* 1994). If, however, the number of binding sites was calculated per weight of CL, then the highest total amount of β-receptors was on d 8–12, *ie* during the height of progesterone secretion. The amount of OT secreted under the influence of NA, increased from d 5 up to d 8 (figs 1, 2). This can be connected with the fact that synthesis of OT prohormone is completed around d 6–7 of the cycle (Fehr *et al,* 1987). Therefore, the absence of an increase in NA-induced OT secretion after d 8, when compared with d 7, is supposedly caused by a decrease in β-receptor concentrations (fmol/mg of protein) compensated by an increase in CL weight (Pesta *et al,* 1994). Thus, if the number of β-receptors and number of luteal cell products are correct, then the plateau of OT secretion is established after d 8. A lack of difference in the amount of OT secreted by NA on d 8–10 in this study and on d 11 and 12 (Kotwica and Skarzynski, 1993) confirms this supposition. In bovine CL there are 4–6 d delay between maximal OT-mRNA and luteal OT concentrations. OT gene transcription takes place during the first days of the cycle (Ivell *et al,* 1985). Electron microscopic studies revealed that neurophysin immunoreactive dense granules are already formed on d 3 of the estrous cycle (Fields and Fields, 1986), and their number reaches a peak around d 11, just when luteal concen-

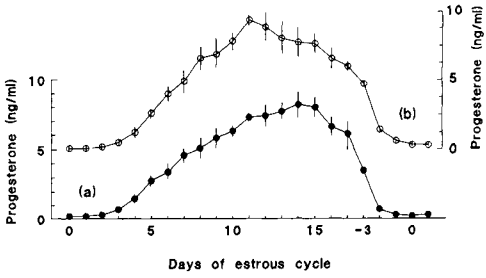

Fig 3. Progesterone concentrations (mean ± SEM) across the estrous cycle in (a) noradrenaline (n = 4) and (b) saline (n = 3) infused heifers on d 5–10 of the estrous cycle.

Table I. Mean (± SEM) oxytocin concentrations (ng/g tissue) on d 10 of the estrous cycle in corpora lutea of heifers treated with saline or noradrenaline (4 mg/30 min/day) on d 5–10.

Hormone	*Treatment*	
	Saline (n = 4)	*Noradrenaline (n = 4)*
Oxytocin (ng/g tissue)	314.6 ± 14.5	169.9 ± 8.1
	372.2 ± 28.1	190.6 ± 28.1
	343.5 ± 23.9	195.4 ± 50.3
	363.0 ± 59.9	
		#06733 289.8 ± 54.8

Details dealing with heifer # 06733 given in *Results;* each value was determined in quadruplicate.

tration of OT is the highest. A rapid increase in bovine CL weight from d 1 to d 7 (Ivell *et al,* 1985) seems to be accompanied by an increase in post-translational processing pathway enzymes. It was shown that activity of peptidyl glycine α-amidating monooxygenase (enzyme processing OT from prohormone) increases from d 2–9 of the estrous cycle and it is continuously high up to d 15 (Sheldrick and Flint, 1989). This is in agreement with data reported by Camier *et al* (1991) that incomplete processing could be responsible for the low level of OT in early bovine CL.

In CL collected from NA-infused heifers on d 10, OT concentrations were more than 50% lower than the control. This, however, did not influence the length of the estrous cycle. Similarly, reduction of ovarian OT content by 75% on d 12 or 16 did not affect the duration of the cycle (Kotwica and Skarzynski, 1993). We therefore suggested that during luteolysis in cattle OT can have only a modulatory and supportive effect on the process which has already started. Data by Fields *et al* (1992) support this view. On the other hand, it was shown that luteolysis depends on the exposure of endometrium on physiological progesterone concentrations during diestrus (Geisert *et al,* 1992) since this time of exposure is crucial for uterine OT receptor synthesis (Morgan *et al,* 1993). Progesterone exposure lasts in cattle for 11–14 d depending on the estrous cycle duration (Hansel and Convey, 1983). Thus, one could suspect that reduction of OT content in the CL in this study would affect progesterone secretion and finally the time of luteolysis. However, nothing like this was observed. Therefore, it is possible that the remaining 50% of OT in the CL fulfills its function successfully, or that the stimulatory effect of NA on progesterone secretion can mask the reduced OT amount.

In the present experiments, significant increases in progesterone levels stimulated by NA were found on d 5–7 (fig 2). It seems that the decrease in β-receptor maximal binding from the early days of the cycle can be compensated by quick growth of the CL. Hence, the total number of β-receptors per CL increases (Pesta *et al,* 1994). Therefore, the supposed progesterone response to NA treatment on d 8–10 in this study was no different from the same response on d 11 and 12 (Kotwica and Skarzynski, 1993).

In conclusion, NA given in the early stage of the estrous cycle depletes the CL of OT by approximately 50%. This, however, does not affect the CL secretory function. Therefore, we assume that if ovarian OT is involved in steroidogenesis it plays only a modulatory role.

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