Mechanism of noradrenaline influence on the secretion of ovarian oxytocin and progesterone in conscious cattle

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Noradrenaline stimulates the concomitant release of ovarian oxytocin and progesterone in cattle within a few minutes, but the mechanism of its action is unknown. Changes in α - and β -receptors and blood pressure were considered as possible mechanisms of the noradrenaline effect. Heifers in group 1 ($n = 4$) were infused with noradrenaline ($0.16 \mu\text{g kg}^{-1} \text{min}^{-1}$) for 30 min into the aorta abdominalis (cranial to the origin of the ovarian artery) on day 10. On days 11 and 12 before noradrenaline, phentolamine (α -blocker; $30 \mu\text{g kg}^{-1} \text{min}^{-1}$) or propranolol (β -blocker; $5 \mu\text{g kg}^{-1} \text{min}^{-1}$) were infused for 30 min. Four other heifers were infused with noradrenaline only as controls. Only propranolol inhibited the stimulatory effect of noradrenaline on the secretion of progesterone and oxytocin. In group 2, heifers ($n = 4$) were infused, making use of the latin square design, with vasoconstrictive (angiotensin; $0.042 \mu\text{g kg}^{-1} \text{min}^{-1}$) or vasodilatory (xanthinol-theophylline nicotinate; $250 \mu\text{g kg}^{-1} \text{min}^{-1}$) drugs that do not act through the adrenoceptors. Noradrenaline ($0.3 \mu\text{g kg}^{-1} \text{min}^{-1}$) was given 1 h later as in group 1. Blood pressure changes were measured in the posterior aorta abdominalis and oxytocin and progesterone concentrations were determined in the blood samples collected from the jugular vein. Noradrenaline and angiotensin increased ($P < 0.01$), whereas xanthinol decreased ($P < 0.01$), blood pressure during their infusion. However, the rise of oxytocin and progesterone concentrations was observed only after noradrenaline infusion. We suggest that (i) noradrenaline enhances the secretion of progesterone and oxytocin from the corpus luteum acting through β -receptors; and (ii) increase of vascular blood pressure which does not occur concomitantly with β -receptor stimulation does not appear to be involved in the corpus luteum secretion.

Introduction

Noradrenaline injected into the aorta abdominalis stimulates the secretion of ovarian oxytocin and progesterone in heifers (Kotwica *et al.*, 1991a), but not if the same dose is infused into the jugular vein (Kotwica *et al.*, 1990). Noradrenaline is therefore considered to act locally upon the ovary. However, the nature of this effect is unknown. The response of granulosa cells to noradrenaline challenge measured by oxytocin or progesterone secretion in studies *in vitro* was observed after a few days (Luck and Jungclas, 1987). It was therefore suggested that such prompt secretion of ovarian hormones in experiments *in vivo* may partly depend upon noradrenaline action through the vascular adrenoceptors and blood pressure changes. This contention is supported by studies by Heap *et al.* (1989), who found that noradrenaline caused a rapid release of oxytocin in ewes, closely associated with ovarian blood flow reduction, probably via vascular bed α -receptors (Reynolds and Ford, 1984). This hypothesis was verified in the present paper.

Materials and Methods

Animals and surgical procedure

Mature heifers of Black and White breed (350–450 kg body weight) with regular oestrous cycles were synchronized by means of a PGF_{2a} analogue (500 μg ; Oestrophon, Spofa). The onset of oestrus was taken as day 0. A catheter was inserted into the posterior aorta abdominalis through the coccygeal artery (Kotwica *et al.*, 1990) on day 10 for infusion of either saline or drugs. The tip of this cannula was positioned cranially to the origin of the ovarian artery. Consequently, the infused drugs could be transported by the bloodstream directly into the reproductive tract. A jugular vein was cannulated for the collection of blood. Furthermore, in Expt 2, heifers had one more catheter placed in the posterior aorta abdominalis through the dorsal costoabdominal artery (Haibel *et al.*, 1989) for measurement of blood pressure.

Preliminary experiments

Kotwica *et al.* (1990, 1991a) found that 4 mg of noradrenaline given for 30 min ($0.3 \mu\text{g kg}^{-1} \text{min}^{-1}$), which is a therapeutic

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dose recommended for humans, was very effective at stimulating the corpus luteum in cattle if it was given into the aorta abdominalis but not if it was infused into the jugular vein. In the study reported here noradrenaline was infused after treating heifers with α - or β -blockers; we wanted to avoid the criticism that the dose of noradrenaline used could overcome the effect of adrenoceptor blockers. Four different doses of noradrenaline (1, 2, 3 or 4 mg) were therefore infused in four different heifers on day 10 of the oestrous cycle.

The doses of drugs that did not act on blood vessels through adrenoceptors were determined by infusing a heifer for 30 min with either 0.5, 1.0 or 2.0 mg of angiotensin (Hypertensin, Ciba-Geigy, Switzerland) and another heifer was infused with either 2, 3, or 4 g of xanthinol (Sadamin, Polfa) in the next preliminary experiment. Heart rate and blood pressure were recorded via a catheter inserted into the dorsal costal abdominal artery.

Experiment 1

Four heifers from group 1 were infused with saline and noradrenaline on day 10 and then in latin square design on days 11 and 12 of the oestrous cycle, and were given phentolamine (α -blocker; $30 \mu\text{g kg}^{-1} \text{min}^{-1}$) or propranolol (β -blocker; $5 \mu\text{g kg}^{-1} \text{min}^{-1}$) for 30 min and then 2 mg of noradrenaline also for 30 min. Every subsequent noradrenaline infusion causes a lower secretion of oxytocin (Skarzynski *et al.*, 1991; Kotwica and Skarzynski, 1993), in accordance with the proposal that once the synthesized peptide has been released this store cannot be replenished by synthesis *de novo* (Ivell, 1987), but only by using prohormone pools. Four other heifers (group 2) were therefore infused on day 10, 11 and 12 with saline and this was followed by noradrenaline as in group 1. Concentrations of oxytocin and progesterone in these heifers served as control values and were compared with those in group 1.

Experiment 2

Four heifers were infused for 30 min on days 11 and 12, using the latin square design, with vasoconstrictive (0.5 mg angiotensin i.e. $0.042 \mu\text{g kg}^{-1} \text{min}^{-1}$) or vasodilatory (3.0 g xanthinol; $250 \mu\text{g kg}^{-1} \text{min}^{-1}$) drugs. One hour after the infusion of drug was terminated, 2 mg of noradrenaline was given as in group 1. Blood pressure changes in the posterior aorta abdominalis were recorded and oxytocin and progesterone concentrations in the plasma of blood collected from jugular vein were determined every 5–10 min.

Hormone determination

Oxytocin and progesterone were determined as described by Schams *et al.* (1979) and Kotwica *et al.* (1990), respectively. Ovine progesterone antiserum (GDN No 337, kindly donated by G. D. Niswender, Colorado) was characterized as described by Gibori *et al.* (1977) and rabbit oxytocin antiserum (R-1, donated by G. Kotwica) showed less than 0.01% crossreactivity with arginine-vasopressin, lysine-vasopressin, angiotensin, vasotocin and somatostatin (Sigma, Deisenhofen). Sensitivity of the assay averaged $0.3\text{--}0.6 \text{ ng ml}^{-1}$ for progesterone and 3 pg ml^{-1} for oxytocin. The coefficients of correlation between added and

Fig. 1. Plasma (a) progesterone and (b) oxytocin before, during (—) and after infusion of different doses of noradrenaline (●) 4 mg (▲) 3 mg (○) 2 mg (△) 1 mg into the aorta abdominalis in one heifer on day 10 of the oestrous cycle.

Fig. 2. Influence of different doses of (a) xanthinol (XAN) in one heifer and (b) angiotensin (ANG) in another heifer infused into the aorta abdominalis on the peripheral concentrations of progesterone (●), oxytocin (○) and arterial blood pressure (▲). One hour after the final infusion of each drug, 4 mg of noradrenaline (NA) was infused. Horizontal bars illustrate drug infusion lasting 30 min each.

Fig. 3. Effect of noradrenaline (NA; 2 mg) infused into the aorta abdominalis of one representative heifer pretreated with (a) saline, (b) phentolamine (PHENT; 30 $\mu\text{g kg}^{-1} \text{min}^{-1}$) and (c) propranolol (PROP; 5 $\mu\text{g kg}^{-1} \text{min}^{-1}$) on day 10, 11 and 12 respectively, on the peripheral concentrations of progesterone (●) and oxytocin (○).

recovered amounts of progesterone and oxytocin in plasma for four different concentrations were 93 and 95%, respectively. Intra- and interassay coefficients of variation were 9.6 and 20.2% for progesterone and 5.6 and 10.5% for oxytocin, respectively, and depended on hormone concentrations.

Statistical analysis

Mean values of hormones for each period of blood sampling during treatment were compared with the previous concentration by a one-way analysis of variance, to estimate the time of the significant rise in the concentration of a hormone after treatment. The areas under the curves of oxytocin and progesterone concentrations were calculated during treatment with xanthinol, angiotensin and noradrenaline. Differences between mean values ($\pm$ SEM) were estimated by one-way analysis of variance.

Results

Preliminary experiments

There was a dose-dependent effect of noradrenaline on oxytocin and progesterone release ($P < 0.01$) (Fig. 1). The dose of 2 mg of noradrenaline was therefore chosen for further studies.

Infusion of the highest dose of angiotensin was interrupted owing to a violent rise of blood pressure from 16.5 kPa of up to 24.0 kPa (180 mm Hg within a few minutes and it rose continuously). The doses of 0.5 mg angiotensin and 3 mg xanthinol were therefore chosen for further studies (Fig. 2).

Experiment 1

Noradrenaline given after pretreatment of heifers with saline or phentolamine (α -blocker) stimulated ($P < 0.01$) the secretion of both progesterone and oxytocin within a few minutes. Only propranolol given before noradrenaline infusion prevented its stimulatory effect (Fig. 3; Table 1). Furthermore, the comparison of oxytocin and progesterone concentrations stimulated by noradrenaline on days 10–12 after adrenoceptor blockers to those after saline on the same days (Fig. 4) showed that hormone concentrations were significantly lower only after propranolol pretreatment (Table 1).

Experiment 2

Basal values of blood pressure were in the range 16.0–17.3 kPa (120–130 mm Hg). Infusion of heifers with angiotensin increased blood pressure from a basal value of 16.0–17.3 kPa up to 24.0–24.7 kPa ($P < 0.01$), whereas xanthinol decreased blood pressure by 17–25% to 13.0–13.3 kPa during treatment ($P < 0.05$). The increase of blood pressure caused by noradrenaline infusion was similar ($P > 0.05$) to that shown by angiotensin. In spite of these blood pressure changes, only noradrenaline could stimulate oxytocin and progesterone secretion significantly (Table 2; Fig. 5).

Discussion

A prompt response of oxytocin and progesterone to noradrenaline challenge was observed in this study and in earlier work (Kotwica *et al.*, 1991a), contrary to the response of granulosa cells to catecholamines which lasts for hours or even days in an *in vitro* system (Luck and Jungclas, 1987). We have previously assumed (Kotwica *et al.*, 1991a) that the response was caused by the direct action of noradrenaline on blood vessels and on myofibrils of smooth ovarian muscles. However, present data showed that the blood pressure changes lasting for a short period, without simultaneous activation of luteal β -receptors, had no influence on the secretory function of the corpus luteum (Table 2; Figs 2 and 5). The increase in blood pressure caused by angiotensin even produced a transient decrease of progesterone release (Table 2). The early corpus luteum is devoid of innervation (Bahr *et al.*, 1974; Burden, 1978) but essential ingrowth of adrenergic nerves is observed during the luteal phase in humans (Hamberger *et al.*, 1980) and is suggested to be associated with the formation of blood vessels in the developing corpus luteum. However, sympathetic denervation of the rat ovary does not influence ovarian blood flow (Gibson and Roche, 1986). However, since noradrenaline (Battista *et al.*, 1987; Heap *et al.*, 1989; Kotwica *et al.*, 1991a) and β -mimetics (Condon and Black, 1976; Wheeler *et al.*, 1988; Kotwica *et al.*, 1991b) influence the secretion of the corpus luteum, we suggest

Table 1. Progesterone and oxytocin concentrations above the baseline in the peripheral blood (area under the curve; mean $\pm$ SEM) during infusion of noradrenaline (2 mg for 30 min) on days 10, 11 and 12 in heifers pretreated in latin square design with saline, phentolamine or propranolol and in control heifers pretreated with saline only

Hormone	Group	Days of oestrous cycle		
		10	11	12
Progesterone	Experimental (<i>n</i> = 4)	3.24 $\pm$ 0.60	4.48 $\pm$ 1.17	0.51 $\pm$ 0.30*
	Control (<i>n</i> = 4)	3.46 $\pm$ 0.70	4.02 $\pm$ 0.40	5.60 $\pm$ 1.40
Oxytocin	Experimental (<i>n</i> = 4)	18.35 $\pm$ 1.95	11.32 $\pm$ 1.27	1.05 $\pm$ 0.67*
	Control (<i>n</i> = 4)	18.53 $\pm$ 2.34	9.72 $\pm$ 1.50	9.85 $\pm$ 1.25

*Value significantly different from control (*P* < 0.001).
Number of heifers given in parentheses.

Fig. 5. Concentrations of oxytocin (○) and progesterone (●) in peripheral blood and blood pressure (▲) measured in the castoabdominal artery during infusion of (a) vasodilatory (xanthinol: XAN; 3 g) or (b) vasoconstrictive (angiotensin: ANG; 0.5 mg) drug, followed with 4 mg of noradrenaline (NA). Each drug was infused for 30 min (horizontal bar).

Fig. 4. Influence of noradrenaline (2 mg) infused into the aorta abdominalis (—) of one representative heifer on days (a) 10, (b) 11 and (c) 12, pretreated on each day with saline only (horizontal line) on the peripheral concentrations of progesterone (●) and oxytocin (○).

that the main role of ovarian sympathetic innervation is to act directly upon the secretory part of the ovary and thus modify its function. Data by Martensson and Carter (1982) and by

Wiltbank *et al.* (1990), who found that blood vessels of the corpus luteum do not undergo autoregulation, support this view. Taking into account the early stages of corpus luteum formation and its importance in early pregnancy, this mechanism protects and even supports the normal function of the corpus luteum regardless of blood pressure changes in the general circulation. This seems to be crucial, especially in stressful situations, in domesticated and even more importantly in wild animals, for example during the escape reaction.

Table 2. Mean ($\pm$ SEM) progesterone and oxytocin concentrations above the baseline (area under the curve; cm^2) after 30 min infusion of noradrenaline preinfused with angiotensin or xanthinol in latin square design on days 11 and 12

Hormone	Noradrenaline (4 mg)	Angiotensin (0.5 g)	Xanthinol (3 g)
Progesterone	7.2 ± 2.9	-2.62 ± 1.0	0
Oxytocin	9.0 ± 2.4	0.28 ± 0.26	0

Noradrenaline is involved in LH secretion (Parvizi and Ellendorff, 1982) which is luteotrophic in cattle (Hoffman *et al.*, 1974). However, this was not the case in this study, as the dose of drug infused into the aorta abdominalis did not influence the secretion by the ovary if it was given systemically (Kotwica *et al.*, 1990). Furthermore, LH concentrations were unchanged if measured in blood samples collected from heifers in preliminary experiments during noradrenaline infusion, compared with the pretreatment period (J. Jaroszewski and J. Kotwica, unpublished). This result confirms our assumption that the dose of noradrenaline used exerts a local effect upon the corpus luteum. Isoproterenol (β -mimetic) stimulates progesterone secretion in small but not in large luteal cells (Niswender *et al.*, 1985), suggesting that β -receptors occur primarily on small cells. Furthermore, small but not large cells also seem to possess specific receptors for oxytocin (Niswender *et al.*, 1985; Miyamoto and Schams, 1991). Thus in the present experiment noradrenaline stimulated the β -receptors of the small cells, but caused the release of oxytocin, which is synthesized in large luteal cells (Guldenaar *et al.*, 1984; Fields *et al.*, 1992). We do not know the identity of the signal sent from small to large cells that evokes release of oxytocin simultaneously with progesterone or even before that. Nevertheless we assume that consideration of cell-to-cell communication is crucial for further understanding of the mechanism of noradrenaline influence on the secretory function of the bovine corpus luteum.

Hirst *et al.* (1986) found that depolarization of cells in luteal slices with excess potassium or supplementation of medium with calcium or calcium ionophore caused a rapid onset of oxytocin secretion. These data indicate that oxytocin release is calcium dependent, which is consistent with the exocytotic release of this hormone. It is possible that intracellular products of luteal cells like insulin-like growth factor 1, insulin or prostaglandins (Schams *et al.*, 1988; McArdle and Holtroff, 1989; McArdle 1990), which are affected by noradrenaline, are involved in these processes.

In conclusion, we suggest that (i) constant sympathetic stimulation causes activation of β -adrenoceptors of luteal cells by noradrenaline and through cAMP mediation (Lefkowitz and Caron, 1987) influences the basal progesterone secretion (Kotwica *et al.*, 1991b); (ii) during short-term stress, activation of luteal β -receptors by noradrenaline seems to be amplified by its simultaneous influence on blood pressure that may be attributed to large increases in blood flow to the corpus luteum (Wiltbank *et al.*, 1990) allowing the use of serum-derived lipoprotein as a source of cholesterol for steroidogenesis (Grummer and Carroll, 1988).

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