Intrinsic spontaneous activity and β -adrenoceptor-mediated tubal dilatation affect ovum transport in the oviduct of the cow

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Summary. Oviducts of cows were obtained during the proliferative and secretory phases of the oestrous cycle (determined by macroscopic appearance of the ovaries). Consistently higher frequencies of contraction were observed in the isthmus than in the ampulla, whereas no significant difference was observed between longitudinal and circular specimens, or throughout the oestrous cycle. Basal activity was inhibited by removal of extracellular Ca^{2+} and by verapamil (10^{-5} M). Addition of Ca^{2+} restored activity in the first case, but not in the second. Administration of various adrenoceptor agonists and antagonists revealed that the responses of the longitudinal and circular smooth muscles were primarily mediated by β -adrenoceptors ($\beta_2 > \beta_1$), while the α -adrenoceptor-mediated contractions (α_2 -subtype) were masked by the marked β -adrenergic dominance. The results support the concept that the significance of adrenergic nerves in the cow oviduct may be to produce relaxation rather than contraction; the combined β_1 - and β_2 -adrenoceptor-mediated tubal dilatation and the intrinsic spontaneous activity seem to be the main factors affecting ovum/embryo transport throughout the oviduct in cows.

Keywords: adrenoceptors; cattle; oviduct; smooth muscle; spontaneous activity

Introduction

The oviduct is engaged in various processes essential for reproduction. Within the ampulla the egg is transported in a complex to-and-fro movement (Verdugo *et al.*, 1976) influenced by the motility of the cilia and secondarily by smooth muscle activity (Arnold & Shorey, 1985). The tube-locking mechanism of the ampullary–isthmic junction (AIJ), as well as the rapid passage into the uterus, seem to be effected by tubular muscular activity (Anand & Guha, 1978), under the control of the sympathetic nervous system (Brundin, 1965; Pauerstein *et al.*, 1974).

The adrenergic response has been analysed in several animal species. α -Adrenergic receptors predominate in the oviduct musculature of the rabbit (Howe & Black, 1973) and pig (Rodríguez-Martínez, 1984). However, in the human Fallopian tube, β -adrenoceptors predominate throughout the menstrual cycle, and stimulation of adrenergic nerves would therefore produce relaxation more than contraction (Samuelson & Sjöstrand, 1986). However, the physiological function of genital tract β -receptors is controversial, because noradrenaline, which is by far the predominant catecholamine in the myosalpinx, is among the weakest of catecholamines in stimulating β -adrenoceptors (Jansen, 1984).

This discrepancy of results among species has motivated our study of the characteristics of the spontaneous activity of the oviduct of the cow and the influence of adrenergic receptors over it. Our aim was to clarify the possible role of these receptors in regulating ovum/embryo transport.

Materials and Methods

Tissues. Oviducts with their corresponding ovaries from non-pregnant sexually mature cows were collected at the local slaughterhouse and transported to the laboratory in ice-cold Krebs' solution (see below) within 30 min. Reproductive status, determined by macroscopic examination of the ovaries, was designated as proliferative or secretory according to Rosenberger (1969).

Oviducts were transferred to a Petri dish containing Krebs' solution at room temperature, and carefully dissected from adjacent tissues. Longitudinal strips (10 mm long, 2–4 mm width) and rings (3–4 mm width) from the middle portions of the ampulla (longitudinal and circular ampulla) and isthmus (longitudinal and circular isthmus) were prepared according to the methods previously described by Gimeno *et al.* (1984) for pigs, and Helm *et al.* (1982a) for human tissue.

Preparations were mounted in 30-ml organ baths containing Krebs' solution at 37°C bubbled with a mixture of 5% CO₂ and 95% O₂.

Attachment to the bath and connections to the transducer (Grass FTO3C) were by direct silk ties for longitudinal strips, or by stainless-steel hooks for circular ones. Changes in isometric force were recorded on a Grass polygraph (model 79D).

The longitudinal and circular ampullary and isthmus preparations were given initial passive tensions of 1.5 g, 1 g, 1 g and 0.75 g respectively. At these tensions, determined in previous experiments, the preparations showed uniform rhythmic spontaneous activity.

After equilibration (60–90 min), the frequency of spontaneous contractions was measured during a 5-min period. Cumulative dose–response curves were performed for the different agonists, expressing the effect of each agonist as a percentage of the maximum response in each curve. Antagonists were added 20 min before the next dose–response test, and their effects were expressed as a percentage of the control, i.e. with the agonist.

Statistics and calculations. The drug concentration eliciting half maximum contraction (EC₅₀) or relaxation (IC₅₀) was determined graphically for each curve by linear interpolation. Values are expressed as the mean $\pm$ s.e.m., and statistical analyses were performed by Student's *t* test and analysis of variance. The level of statistically significant difference was considered to be $P < 0.05$.

Solutions. Normal Krebs' solution had the following composition (mmol/l): NaCl 119, NaHCO₃ 24.9, KCl 4.6, MgCl₂(6H₂O) 1.2, KH₂PO₄ 1.4, EDTA(2H₂O) 0.0027, glucose 11, and CaCl₂ 1.5. In experiments involving a calcium-free solution, CaCl₂ was omitted from the normal Krebs' solution and replaced with 0.01 mM-EGTA.

Substances. The following substances were purchased: D-L-Noradrenaline HCl (Serva Feinbiochemica GmbH & Co., Heidelberg, FRG), phenylephrine HCl (Sigma Chemical Co., St. Louis, MO), B-HT-920 (Dr K. Thomae GmbH, Biberach an der Riss, FRG), isoprenaline sulphate (C. H. Boehringer Sohn, Ingelheim am Rhein, FRG), salbutamol sulphate (Glaxo Labs., Ltd, Greenford, Middlesex, UK), phenoxybenzamine HCl (Smith Kline & French, Welwyn Garden City, UK), yohimbine HCl (Sigma), propranolol HC and practolol (ICI-Pharma Arzneimittelwerk, Plankstadt, FRG), and butoxamine HCl (The Wellcome Foundation, London, UK).

Results

All preparations exhibited a standard rhythmic motility with uniform and symmetrical contractions throughout the experiments (Fig. 1). Isthmic preparations showed significantly higher frequencies of contraction ($P < 0.01$) than did the ampullary preparations. However, no significant differences between frequencies of contraction in longitudinal and circular preparations from the ampulla and isthmus, in the proliferative or secretory phases of the oestrous cycle, could be detected. No cyclical difference in the frequency of contraction could be observed in any of the 4 preparations studied (Table 1).

Calcium withdrawal from the solution abolished in approximately 20 min the spontaneous activity in all 4 preparations. Subsequent administration of cumulative concentrations of calcium, caused the quick recuperation of the activity, even with minimum quantities of this ion (0.5 mM-Ca²⁺) (Fig. 2a). Verapamil (10⁻⁵ M) completely inhibited the spontaneous activity in a few minutes; the motility of the oviduct was not restored with the addition of increasing concentrations of calcium (Fig. 2b).

Noradrenaline (10⁻⁷–10⁻⁴ M) elicited dose-dependent relaxations in all 4 preparations studied (with an order of potency of longitudinal ampulla > circular isthmus > circular ampulla > longitudinal isthmus). Phenoxybenzamine (10⁻⁶ M) shifted to the left ($P < 0.01$) the relaxing control

Table 1. Frequency of spontaneous contractions in oviduct preparations of cattle in the proliferative and secretory phases of the oestrous cycle

Preparation	Contractions/min	
	Proliferative phase	Secretory phase
Ampulla		
Longitudinal	4.82 ± 0.4 (18)	5.61 ± 0.3 (18)
Circular	4.73 ± 0.2 (18)	5.12 ± 0.3 (18)
Isthmus		
Longitudinal	6.72 ± 0.2* (18)	6.91 ± 0.3* (18)
Circular	7.59 ± 0.3* (18)	6.59 ± 0.4* (18)

Values are mean ± s.e.m. for the no. of preparations in parentheses. * $P < 0.01$ compared with same muscle type in the ampulla.

curves of noradrenaline. Addition of isoprenaline (10^{-8} – 10^{-5} M) evoked a dose-dependent relaxing effect, with no significant difference (expressed as $-\log IC_{50}$ values) between preparations. Propranolol (10^{-6} M) minimized the relaxing effects of isoprenaline ($P < 0.001$). Practolol (10^{-5} M), a selective β_1 -adrenoceptor antagonist, slightly modified the control curves of isoprenaline ($P < 0.05$), but prior administration of butoxamine (10^{-5} M), a β_2 -adrenoceptor blocker, substantially reduced ($P < 0.001$) the effect of isoprenaline. Salbutamol (10^{-8} – 10^{-5} M), a selective β_2 agonist, mimicked the isoprenaline-induced effect in all 4 preparations tested. Pretreatment with butoxamine (10^{-5} M) abolished significantly ($P < 0.001$) the inhibitory effects of salbutamol (Table 2).

The response to noradrenaline was reversed by 10^{-6} M-propranolol, and all tubal preparations responded with dose-dependent contractions (Table 3). Practolol (10^{-5} M) mimicked this effect of propranolol when administered before a single dose of noradrenaline (10^{-5} M) (Fig. 3). To clarify the α -adrenergic component of noradrenaline, phenylephrine and B-HT-920 were used as selective α_1 and α_2 agonists, respectively. Phenylephrine (10^{-7} – 10^{-4} M) had no effect on any of the preparations studied. B-HT-920 originated dose-dependent contractile effects when added to the organ bath at cumulative concentrations of 10^{-7} to 5×10^{-4} M, with no significant difference (expressed as $-\log EC_{50}$ values) between preparations. The contractile effect of B-HT-920 was selectively blocked by yohimbine (10^{-5} M) ($P < 0.001$) (Table 3).

Discussion

Schilling (1962) has described the distribution of muscle layers in ungulates as spirals around the tube, which are less extended in the ampulla than in the isthmus. The fact that the same type of uniform rhythmic spontaneous activity was found in all tubal preparations (Fig. 1), and that no significant difference in frequency of contraction could be observed between longitudinal and circular preparations (Table 1), could be, from a functional point of view, in accordance with Schilling's (1962) theory. The different frequencies of contraction observed between the ampullary and isthmic preparations (Table 1) could be related to differentiated smooth muscle cell membrane properties of these two segments, as discussed by Lindblom *et al.* (1979b) for the human Fallopian tube. Nevertheless, conclusions from the study of frequencies of contraction should be drawn with caution, since the smooth muscle of the oviduct responds actively to stretch (Arjamaa, 1984; Samuelson & Sjöstrand, 1986).

Fig. 1. Spontaneous activity of longitudinal ampulla (LA), circular ampulla (CA), longitudinal isthmus (LI) and circular isthmus (CI) preparations during the proliferative (Pro) and secretory (Sec) phases of the oestrous cycle in cattle.

Fig. 2. Effects on the spontaneous activity of the cattle circular isthmus preparation of (a) calcium withdrawal (0 Ca^{2+}) and restoration; and (b) verapamil (10^{-5} M) treatment and restoration of Ca^{2+} .

Fig. 3. Effect of practolol (10^{-6} M) on the noradrenaline-induced (10^{-5} M) activity (NA) in a circular isthmus preparation of the cattle oviduct.

Table 2. Relaxing effects (expressed as $-\log IC_{50}$ values) of noradrenaline, isoprenaline and salbutamol in the presence and absence of antagonists on oviduct preparations from cattle

Preparation	Noradrenaline	Phenoxybenzamine (10^{-6} M)	+ noradrenaline	Isoprenaline	Propranolol (10^{-6} M)	+ isoprenaline	Practolol (10^{-5} M)	+ isoprenaline	Butoxamine (10^{-5} M)	+ isoprenaline	Salbutamol	Butoxamine (10^{-5} M)	+ salbutamol
Ampulla													
Longitudinal	5.38 ± 0.2 (6)		6.64 ± 0.1^a (4)	7.05 ± 0.3 (8)	4.62 ± 0.2^b (4)		6.90 ± 0.1^c (4)		4.42 ± 0.3^b (4)		6.89 ± 0.3 (6)	3.23 ± 0.4^d (4)	
Circular	5.28 ± 0.1 (6)		6.60 ± 0.3^a (4)	7.09 ± 0.1 (8)	4.05 ± 0.2^b (4)		6.83 ± 0.1^c (4)		4.38 ± 0.2^b (4)		6.51 ± 0.3 (6)	3.05 ± 0.1^d (4)	
Isthmus													
Longitudinal	5.11 ± 0.1 (6)		6.56 ± 0.1^a (4)	7.14 ± 0.2 (8)	4.10 ± 0.4^b (4)		6.96 ± 0.2^c (4)		5.03 ± 0.1^b (4)		6.69 ± 0.4 (6)	3.37 ± 0.3^d (4)	
Circular	5.29 ± 0.1 (6)		6.78 ± 0.2^a (4)	7.18 ± 0.1 (8)	4.73 ± 0.1^b (4)		6.60 ± 0.1^c (4)		4.56 ± 0.3^b (4)		6.64 ± 0.2 (6)	3.50 ± 0.2^d (4)	

Values are mean $\pm$ s.e.m. for the no. of preparations in parentheses.
^a $P < 0.01$, compared with values for noradrenaline alone.
^b $P < 0.001$, ^c $P < 0.05$, compared with values for isoprenaline alone.
^d $P < 0.001$, compared with values for salbutamol alone.

Table 3. Contractile activity (expressed as $-\log EC_{50}$ values) of noradrenaline and B-HT-920 in the presence and absence of antagonists in oviduct preparations from cattle

Preparation	Propranolol (10^{-6} M)+ noradrenaline	B-HT-920	Yohimbine (10^{-5} M)+ B-HT-920
Ampulla			
Longitudinal	5.21 ± 0.3 (4)	5.50 ± 0.3 (6)	$3.05 \pm 0.5^{***}$ (4)
Circular	5.73 ± 0.3 (4)	5.52 ± 0.2 (6)	$3.26 \pm 0.3^{***}$ (4)
Isthmus			
Longitudinal	5.76 ± 0.2 (4)	5.71 ± 0.1 (6)	$3.30 \pm 0.3^{***}$ (4)
Circular	5.16 ± 0.3 (4)	5.29 ± 0.1 (6)	$3.06 \pm 0.4^{***}$ (4)

Values are mean $\pm$ s.e.m. for the no. of preparations in parentheses.

*** $P < 0.001$ compared with other values for that preparation.

It has been argued that the spontaneous activity and sympathomimetic responses of the human tubal smooth muscle vary in relation to different stages of the menstrual cycle, as a reflection of steroid receptor activity (Lindblom *et al.*, 1979a; Batra *et al.*, 1980; Helm *et al.*, 1982a). In our studies, neither quantitative nor qualitative differences in spontaneous contractile activity or reactions to drugs were observed in preparations obtained at different stages of the oestrous cycle in cows. However, this influence should not be excluded because the qualitative method used to classify the oestrous cycle phase does not provide information on accurate hormonal concentrations in the oviduct around the time of ovulation.

Oviducal smooth muscle is a highly excitable tissue that may exhibit spontaneous myogenic activity, and frequency of spike discharge determines the pattern of mechanical activity (Arjamaa, 1982; Bülbring & Tomita, 1987). In these tissues the mechanical activity is assumed to be caused by the occurrence of action potentials or membrane depolarization (Casteels & Phil, 1980). According to Bolton (1979), such membrane depolarization will result in an increased influx of Ca^{2+} , leading to an activation of the contractile proteins. This increase of membrane permeability for Ca^{2+} can be due to the opening of potential-sensitive calcium channels (POCs). These channels open as the potential across the membrane is reduced, and are normally responsible for the upstroke of the action potential in those tissues (highly excitable muscles). Various compounds, the so-called 'calcium channel blockers' (i.e. verapamil), block the entry of Ca^{2+} mainly through the POCs (Fleckenstein, 1977; Bolton, 1979).

In our experiments Ca^{2+} withdrawal abolished, in a few minutes, the spontaneous activity. After addition of increasing concentrations of Ca^{2+} , motility was recovered (Fig. 2a), indicating the extracellular Ca^{2+} -dependence of this basal activity. On the other hand, the strong verapamil-induced effect (Fig. 2b) suggests that POCs are involved in the Ca^{2+} influx supporting the oviducal basal tone.

In this study, both α - and β -adrenoceptor mediated responses were found in all tubal preparations. Noradrenaline elicited dose-dependent relaxing effects, which were reinforced by prior addition of α -adrenergic blockers (Table 2). The fact that noradrenaline evoked relaxation suggests the predominance of β -adrenoceptors in the oviduct of the cow.

β -Adrenoceptors in the genital tract are subcategorized as β_2 -receptors (Lefkowitz, 1976; Jansen, 1984). The findings of our study show that the isoprenaline-relaxing effect was blocked with propranolol and butoxamine ($P < 0.001$), suggesting a β_2 predominance (Table 2).

Except for the early reports of Kendle & Lam Shang Leen (1976), in which a β_1 -receptor population was described in the circular muscle layer of the rabbit oviduct, there is no evidence of such receptors in other species. Our results show that practolol (β_1 -adrenoceptor blocker) shifted the $-\log IC_{50}$ values of isoprenaline significantly ($P < 0.05$) (Table 2). Since noradrenaline is less effective on β_2 -receptors (Lands *et al.*, 1967), and the blockade of β -receptors as well as the β_1 -subtype specifically transformed the noradrenaline-induced relaxation into contraction (Table 3; Fig. 3), we suggest that at least a small population of β_1 -adrenoceptors should be taken into account in the cattle oviduct.

It has been suggested that both subtypes of α -adrenoceptors occur in the human ampullary-isthmic junction, each one predominating during different parts of the menstrual cycle (Helm *et al.*, 1982b). Selective stimulation of α_2 -adrenoceptors, but not those of the α_1 -subtype, mimicked, to the same extent, the noradrenaline-induced responses in the presence of propranolol (Table 3). Furthermore, the selective α_2 -antagonist, yohimbine, evoked a significant ($P < 0.001$) blocking effect on the B-HT-920-induced responses. For this reason, we suggest that, in the oviduct of the cow, the α -adrenergic component involved in tubal contractility would be the α_2 -subtype. This suggestion differs from that formulated by Helm *et al.* (1982b), but our studies were conducted only on ampullary and isthmic tissues since there is no morphological evidence of an ampullary-isthmic junction in the cow. Unfortunately, our investigations were not designed to answer the question whether a local adrenergic sphincter, modulated by sex steroids, as suggested for man (Lindblom *et al.*, 1979b; Helm *et al.*, 1982b), could be involved in ovum transport along the cattle oviduct.

Nevertheless β -adrenergic inhibition was the predominant response of the ampullary and isthmic muscle cells of the cattle oviduct. From a physiological point of view, therefore, the significance of the adrenergic nerves in the cattle oviduct may be to produce relaxation rather than contraction, as Samuelson & Sjöstrand (1986) pointed out for the human Fallopian tube. In this sense, both human and cattle oviducts differ from the rabbit (Howe & Black, 1973) and pig (Rodriguez-Martinez, 1984) in which α -receptors predominate. These interspecies differences should be considered when general assumptions on the control of fertility are made.

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