Responses of the bovine infundibulum to noradrenaline during the oestrous cycle

R. Friedman1*, T. Lais2, D. W. Weber1 and F. Stormshak1†

Department of 1Animal Sciences and 2College of Pharmacy, Oregon State University, Corvallis, OR 97331-6702, USA

An experiment was conducted to investigate the responses of the bovine infundibulum to noradrenaline in vitro. Twelve beef heifers were killed and examined in equal numbers during pro-oestrus and dioestrus (day 12 of the oestrous cycle). A jugular blood sample was collected from each heifer immediately before killing, and the sera were subsequently analysed by radioimmunoassay for progesterone and oestradiol. A strip of each infundibulum (ipsilateral and contralateral to the ovary bearing the largest follicle or functional corpus luteum) was placed into a tissue bath and sequentially exposed to increasing concentrations of noradrenaline (0.4, 0.8 and 1.6 μg ml⁻¹). Changes in frequency and amplitude of isometric contractions and tissue tone were recorded. Infundibula ipsilateral to ovaries bearing the largest follicle in pro-oestrous heifers responded to all concentrations of noradrenaline with greater frequency of contractions than did either contralateral infundibula or both ipsilateral and contralateral infundibula of dioestrous animals (stage of cycle × tissue location interaction, P < 0.01). Changes in the amplitude of contractions induced by noradrenaline did not differ between stages of the cycle studied or the location of the infundibulum within the animal. Mean tone of ipsilateral and contralateral infundibula of pro-oestrous and dioestrous animals increased linearly with exposure to all concentrations of noradrenaline tested (P < 0.05). However, the response of infundibula of dioestrous animals was greater than that of infundibula of pro-oestrous heifers (P ≤ 0.06). These data demonstrate that the bovine infundibulum is responsive to noradrenaline, and suggest that responses to this neurotransmitter may be governed by concentrations of oestrogen and progesterone to which the infundibulum is exposed.

Introduction

The segment of the oviduct proximal to the ovary consists of the funnel-shaped infundibulum, which serves to capture and transport ova to the ampulla of the oviduct. Ovum reception by the infundibulum is a critical step in the sequence of events leading to the reproduction of viviparous mammals. In rabbits and humans, contractile activities of the mesosalpinx and mesovarium as well as rhythmic contractions of the fimbriae contribute to ovum capture (Westman, 1926; Ertel, 1947; Blandau, 1969). Much less is known about the active role of the infundibulum in ovum transport in other species that lack an ovarian bursa.

Oviducts of mammals are innervated primarily by sympathetic nerves, with a possible contribution by the parasympathetic system in some species (Brundin, 1965; Black, 1974; Paton et al., 1977). Adrenergic neurotransmitters and agonists as well as stimulation of sympathetic nerves evoke a marked response by smooth muscle of the isthmus and ampulla (Black, 1974; Johns and Coons, 1981). Spontaneous activity and neurotransmitter-induced responses of isthmic and ampullary smooth muscle have also been shown to be affected by oestrogen and progesterone (Howe and Black, 1973; Higgs and Moawed, 1974; Kennedy and Marshall, 1977; Lindblom et al., 1980).

Although responses of the isthmus and ampulla to neurotransmitters and ovarian hormones have been extensively studied, comparatively little is known about the role of these agents in regulating the activity of the infundibulum. Sterin-Speziale et al. (1978) reported that noradrenaline increased the frequency and strength of isometric contractions of fimbriae removed from women during the follicular phase to a greater extent than did those of fimbriae removed during the luteal phase of the menstrual cycle. Acetylcholine induced only a small increase in the isometric contractility of fimbriae removed at both stages of the cycle. In a subsequent study from the same laboratory, Borda et al. (1980) found that fimbriae of sows immediately before and after ovulation also responded to noradrenaline with an increase in frequency and strength of isometric contractions. These data suggest that the infundibulum, like the isthmus and ampulla, responds to neurotransmitters and that these responses are affected by ovarian hormones.
The present study was conducted to examine the response of the bovine infundibulum to noradrenaline in vitro during pro-oestrus and the mid-luteal phase of the oestrous cycle. A local effect of endogenous ovarian hormones was also evaluated by comparing neurotransmitter-induced responses of the infundibulum ipsilateral to the ovary bearing the largest follicle (pro-oestrus) or corpus luteum with those of the contralateral infundibulum.

**Materials and Methods**

**Animals**

Twelve 2-year-old nulliparous Hereford × Angus heifers exhibiting normal oestrous cycles of 20.1 ± 0.4 days (mean ± SEM) were used in this study. Oestrus (day 0) was determined by twice daily checks with a vasectomized bull. As heifers were detected in oestrus, they were assigned alternately into two groups. Six heifers were injected i.m. with 25 mg PGF2α than salt (Lutalyse, Upjohn Co., Kalamazoo, MI) on day 8, 9 or 10 and killed 3–4 days later on day 11, 12 or 13 of the cycle. This treatment regimen was used to cause luteal regression and thus ensure that the infundibula of these animals would be exposed to predominantly oestrogen at the time of necropsy. The remaining six heifers were killed on day 12 of the cycle when progesterone is the predominant ovarian hormone secreted. All animals were killed by captive bolt pistol and exsanguination. On the morning of the day when animals were killed, jugular blood samples were taken by venepuncture. Serum was separated and frozen at −20°C until radioimmunoassays for progesterone (Koligian and Stormshak, 1977) and oestradiol were performed (Zelinski et al., 1982). All samples of serum were analysed for progesterone and oestradiol in single assays. The intra-assay coefficients of variation for progesterone and oestradiol were 9.8% and 7.0%, respectively.

Immediately after death, infundibula were carefully removed noting which was ipsilateral and which was contralateral to the ovary bearing the corpus luteum or largest follicle. The corpus luteum was dissected free of the ovarian stroma and weighed. Only the diameter of the largest visible follicle was measured with callipers. Infundibula were transported to the laboratory (within 10 min), immersed in Krebs–Ringer bicarbonate (KRB) (4°C) containing glucose (11 mmol l⁻¹) that had previously been gassed with 95% O₂:5% CO₂ (Umbriet et al., 1959). Infundibulums were maintained in oxygenated KRB–glucose solution (4°C) until used (within 40 min).

**Tissue preparation**

The tissue was placed in a Petri dish containing the same oxygenated KRB–glucose solution used in transport, and a strip of infundibulum parallel to the leading edge of the fimbriae measuring 6–8 mm × 38–40 mm was excised. The segment of infundibulum was placed into a glass tissue bath containing 12 ml of KRB–glucose solution continually gassed with 95% O₂:5% CO₂ and maintained at 37°C. One end of the strip was attached with suture to a Pasteur pipette fashioned into a holder, while the other end was fastened (also by means of a suture) to a micro-scale accessory (Gould, U15) coupled to a force transducer (Gould UC2 universal transducing cell). This transducer was connected to a recorder (Beckman Dynograph RB3) via a strain gauge coupler (Beckman type 9853) and an amplifier (type 474A).

The tissue was adjusted for 15 min to a basal resting tension of 250 mg using a micrometer-mounted adjusting slide (Velmex unislide A1500). An additional equilibration of 10 min was allowed before the treatments began.

Each infundibulum was sequentially exposed to 0.4, 0.8 and 1.6 µg noradrenaline ml⁻¹ of medium, and isometric tissue contractions were measured. After addition of noradrenaline (Breco Co, Palo Alto, CA) to the medium, the tissue was allowed to attain maximum response of contraction or relaxation as measured by changes in milligrams of tension. Data during an interval of maximum response lasting 2 min was compared with that of an interval of 2 min immediately before adding noradrenaline. Response of the infundibulum to each concentration of noradrenaline was followed by washing the tissue in 72 ml KRB–glucose solution for 2–4 min and allowing a further 2–4 min equilibrium period before further treatment. After the infundibula had been subjected to all concentrations of noradrenaline, they were individually weighed, oven-dried for 48 h (70°C) and re-weighed to obtain an estimate of their dry mass.

**Measurements of tissue responses**

The response of the infundibulum to each concentration of noradrenaline was evaluated by determining the frequency and amplitude of spontaneous contraction and mean tissue tone. Changes in the frequency of contraction were determined by obtaining the difference between the number of recorded contractions during the maximum response period of 2 min and those during the equilibration period of 2 min immediately before the particular treatment in question. Changes in the mean amplitude of contractions were determined by measuring the height of each recorded contractile response during the treatment period of 2 min, followed by subtraction of the mean amplitude of contraction observed during the preceding equilibration period of 2 min. Changes in mean tissue tone, a measure of tension in resting tissue, were examined after exposure to each concentration of noradrenaline. A formula similar to that used in calculating mean cardiovascular blood pressure as derived from phasic blood pressure recordings (Rushmer, 1961) was used to determine the difference in mean tissue tone between the 2 min treatment period and the 2 min equilibration period.

$$\bar{x}_T = \bar{x}_B + \bar{x}_A/3,$$

where T is tissue tone, B is base of contraction, and A is amplitude of contraction.

**Statistical analyses**

Data for each characteristic response to noradrenaline were analysed as a factorial-split-plot by analysis of variance. The factors were stage of cycle (pro-oestrus and dioestrus), location of infundibulum (ipsilateral and contralateral) and dose (concentra-
tractions of noradrenaline). Linearity and slope of the dose–response curves for each characteristic were determined by orthogonal comparisons. Data for wet and dry masses of infundibula were also grouped as a $2^2$ factorial and analysed by use of split-plot analysis of variance. Differences in ovarian characteristics and serum hormone concentrations were tested for significance by Student’s unpaired $t$ test.

### Results

Ovarian characteristics and serum concentrations of steroid hormones of all experimental animals are presented in Table 1. Although treatment with prostaglandin to induce luteal regression was effective, only two of six heifers exhibited behavioural oestrus and none of the animals had ovulated before being killed. Mean diameter of the largest follicle was greater in pro-oestrous than in dioestrous animals but the difference was not statistically significant. In all but two animals, the follicle of largest diameter was present in the ovary with the regressing corpus luteum (pro-oestrus) or the functional corpus luteum (luteal phase). As anticipated, systemic serum concentrations of oestradiol were greater ($P < 0.06$) in heifers during pro-oestrus than during the luteal phase of the cycle, while the opposite situation occurred for serum concentrations of progesterone ($P < 0.03$).

The stage of the cycle, more specifically the change in the ratio of progesterone to oestrogen secreted, had no effect on the mass of the infundibulum sample taken. The ipsilateral infundibulum sample did not differ in either wet or dry mass from the contralateral infundibulum sample for either pro-oestrous or luteal phase animals. Mean wet masses of infundibula samples of pro-oestrous and dioestrous heifers were (ipsilateral versus contralateral) 286.0 ± 28.3 versus 267.0 ± 22.4 and 242.4 ± 17.9 versus 224.7 ± 26.6 mg, respectively, while corresponding dry masses were 34.2 ± 2.4 versus 32.4 ± 2.1 and 28.3 ± 2.3 versus 27.8 ± 3.7 mg, respectively. Because masses of infundibula did not differ significantly within or among animals, no adjustment of the data for this characteristic was considered to be necessary before statistical analysis.

Stage of the cycle and location within the animal affected the frequency of contractions of infundibula in response to exposure to noradrenaline in vitro (stage × location interaction; $P < 0.01$). This interaction was caused by the infundibulum ipsilateral to the ovary bearing the largest follicle in pro-oestrous heifers responding to all concentrations of noradrenaline with greater frequency of contractions than did the contralateral infundibulum or both ipsilateral and contralateral infundibula of luteal-phase animals (Fig. 1). Although ipsilateral infundibula of pro-oestrous heifers appeared to respond to increasing concentrations of noradrenaline with a linear increase in frequency of contractions, the overall dose–response relationship for infundibula was not statistically significant.

Amplitude of the contractions of infundibula induced by

### Table 1. Ovarian characteristics and serum hormone concentrations in heifers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pro-oestrus (g)</th>
<th>Luteal (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus luteal mass</td>
<td>0.852 ± 0.074</td>
<td>3.223 ± 0.267*</td>
</tr>
<tr>
<td>Largest follicle, diameter</td>
<td>17.9 ± 1.0</td>
<td>13.7 ± 1.2</td>
</tr>
<tr>
<td>Serum progesterone (ng ml⁻¹)</td>
<td>0.20 ± 0.02</td>
<td>2.31 ± 0.15*</td>
</tr>
<tr>
<td>Serum oestradiol (pg ml⁻¹)</td>
<td>7.88 ± 1.66</td>
<td>4.95 ± 0.31†</td>
</tr>
</tbody>
</table>

Values are means ± SEM and are based on samples from six heifers at each stage of the cycle.

* $P < 0.01$

† $P < 0.05$.

![Fig. 1. Changes in the frequency of spontaneous contractions of bovine infundibula induced by noradrenaline in vitro. Infundibula ipsilateral and contralateral to the ovary bearing the largest follicle or corpus luteum were from six heifers in pro-oestrus (□) or six heifers in mid-dioestrus (■). Each bar represents the mean ± SEM.](image-url)
noradrenaline was similar for all concentrations of noradrenaline studied and did not differ between stages of the cycle or between locations (ipsilateral versus contralateral) within animals (Fig. 2). In contrast, ipsilateral and contralateral infundibula of pro-oestrous and luteal-phase animals responded to all concentrations of noradrenaline with corresponding linear increases in mean tone ($P < 0.05$; Fig. 3). However, mean tone of the ipsilateral and contralateral infundibula during pro-oestrus was less than that of infundibula during the luteal phase of the cycle ($P \leq 0.06$).

**Discussion**

The results of these experiments demonstrate that the bovine infundibulum is responsive to noradrenaline. However, the responses evoked by this neurotransmitter may be governed by the ovarian hormones to which the infundibulum is exposed. Hormones secreted by the bovine ovary can affect the function of distant target tissues via the systemic circulation and can also act locally on tissues of the reproductive tract. Ford et al. (1976) showed that ovarian hormones act locally in cows to alter the contractility of uterine arterial smooth muscle induced by nerve stimulation. Additional support for a local effect of ovarian hormones is provided by the data of Pope et al. (1982), who found that a concentration gradient of progesterone exists in tissues (mesosalpinx and mesometrium) ipsilateral but not contralateral to the ovary bearing the corpus luteum in cows. Experimental results suggest that oestrogen secreted by the ovaries of cows during pro-oestrus may act locally to enhance the contractility of the ipsilateral infundibulum. Frequency of contractions of the infundibulum adjacent to the ovary bearing the largest follicle in pro-oestrous heifers was increased by noradrenaline compared with that of the contralateral infundibulum or the infundibula of animals exposed to progesterone predominantly.

These data are in general agreement with the results of previous investigations demonstrating that the frequency of contractions of oviductal smooth muscle is greater when under the influence of exogenous or endogenous oestrogen than of progesterone (Howe and Black, 1973; Higgs and Moawed, 1974; Spilman, 1974; Gimeno et al., 1976; Sterin-Speziale et al., 1978; Lindblom et al., 1980). Tonic release of noradrenaline from adrenergic nerve terminals in the bovine infundibulum exposed to increased local concentrations of oestrogen may enhance the contractility of this structure and thus promote its ability to capture the ovum. The local pathway by which ovarian steroids are able to reach the infundibulum of cows may be similar to that found in primates by Beachy et al. (1980). These workers demonstrated that tissue attaching the ovary to the adjacent fimbriae contains venous anastomoses between the two organs, as well as being endowed with lymphatics.

During the luteal phase of the cycle, ipsilateral and contralateral infundibula of heifers responded to noradrenaline with an increase in tone compared with that of infundibula from heifers in pro-oestrus. The increased systemic concentration of progesterone to which the contralateral infundibulum was exposed precluded detection of any local effect of the steroid, on the ipsilateral infundibulum. The mechanism by which progesterone enhances the ability of noradrenaline to increase infundibular tone is unknown. Noradrenaline-induced increase

**Fig. 2.** Changes in the amplitude of spontaneous contractions of bovine infundibula after exposure to noradrenaline in vitro. Infundibula ipsilateral and contralateral to the ovary bearing the largest follicle or corpus luteum were from six heifers in pro-oestrus (□) or six heifers in mid-dioestrus (■). Each bar represents the mean ± SEM.

**Fig. 3.** Noradrenaline-induced changes in infundibular tone as determined in vitro. Infundibula ipsilateral and contralateral to the ovary bearing the largest follicle or corpus luteum were from six heifers in pro-oestrus (□) or six heifers in mid-dioestrus (■). Each bar represents the mean ± SEM.
in infundibular tone during progesterone dominance was not due to an increase in amplitude of contractions because this response of the infundibula to the neurotransmitter did not differ between stages of the cycle studied.

It is generally acknowledged that the smooth muscle present in the infundibulum of all mammals is sparse compared with the more distal portions of the oviduct. Reports on the presence of circular or longitudinal smooth muscle in the bovine infundibulum are equivocal. According to Beck and Boots (1974), the bovine infundibulum is characterized by the presence of an inner longitudinal and circular layer of smooth muscle. However, Lombard et al. (1950) reported that smooth muscle was not found in the infundibulum of cows. Thus, the nature of the smooth muscle in the bovine infundibulum that responded to noradrenaline is unknown. Alternatives to typical circular or longitudinal smooth muscle may be the smooth muscle of arterioles or the mesotubarium superius, portions of which may have been included in the samples removed for study. However, this seems unlikely because care was taken to remove adhering ligaments from the samples used for the experiments in vitro.

On the basis of the results of this study, it is proposed that the bovine infundibulum is not merely a passive structure but rather one that undergoes rhythmic contractions, particularly when under the influence of oestrogen, and may thus play an active role in ovum reception.

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