Changes in ovarian contraction by endothelin-2 receptor system in the feline ovary

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Abstract

Endothelin-2 (ET-2) is transiently expressed in the granulosa cells of mammalian periovulatory follicles immediately prior to ovulation. Ex vivo experiments showed that, upon treatment with ET-2, the rodent ovary rapidly contracts. When the endothelin receptor pathway is antagonized in vivo, ovulation is inhibited in rodents. These findings lead us to postulate that ET-2–induced contraction of smooth muscles in the ovarian cortex is a final trigger for follicle rupture at the time of ovulation. Similar to human and other mammalian, feline ovaries possess a layer of contractile smooth muscle-like cells around developing follicles, known as the theca externa. In addition, feline ovaries have been documented to spontaneously contract ex vivo; however, the function of this spontaneous contraction and whether ET-2 induces ovulation remains to be determined. Here, we evaluated the characteristics of feline ovarian contraction using morphophysiological methods in the absence or presence of physiological doses of ET-2. Whole fresh feline ovaries were collected after ovary procedures through the Junior Surgery Program at the College of Veterinary Medicine at the University of Illinois. Of 15 ovaries tested, all demonstrated a period of strong and sustained contraction when washed with a 50 mM solution of feline ET-2 peptide for 30 minutes, with an average increase in baseline force of 2.48 ± 0.40 mN. Additionally, when washed for 30 minutes with 140 mM solution of the dual ET receptor antagonist tezosentan contraction was reduced in a dose-dependent manner. Of these ovaries, 4 demonstrated spontaneous contractions prior to ET-2 treatment, with average amplitudes of 4.82 ± 2.45 mN, duration of 22.2 ± 4.6 sec, and a time of 60 ± 9.2 sec between contractions. These contractions continued after ET-2 treatment, but contraction amplitude was reduced (1.25 ± 0.85 mN) as was the time between contractions (13.8 ± 4.5 sec) for all ovaries. There was no change in the duration of these contractions (20.13 ± 4.66 sec). Measurement of mRNA expression by quantitative PCR reaction showed that feline ovaries express mRNA for ET-2, and both isoforms of endothelin receptors (ET-A and ET-B), and endothelin converting enzymes 1 and 2 (ECE-1 and ECE-2). This study demonstrates that ET-2 produces a feline ovarian cortical contraction. Future work will determine the impact of inhibiting ET-2 and the endothelin receptor pathways in vivo on follicle rupture in the feline ovary.

Introduction

We have previously shown that ET-2 was both transiently and transiently expressed in the granulosa (G3) of periovulatory ovaries immediately prior to ovulatory follicle rupture (1) via a hypoxia-driven pathway (2). When treated in vitro, ET-2 induced an immediate and sustained contraction in the periovulatory ovary via an endothelin receptor A (ET-A)–mediated pathway (3). These findings led us to hypothesize that ET-2 induces follicle rupture by contracting periovulatory follicles as a last moment of control, and ET-2 expression and follicle rupture occur almost simultaneously (4). The shortening of these external cells drives the ovary, cumulus complex, and animal fluid from the ovary into the periovarian space and interstitium. We hypothesize that ET-2 plays critical roles for ovulation via follicular contraction. In this study, we tested a portion of the hypothesis by treating whole feline ovaries with ET-2 and ET-receptor antagonists and quantifying their transient response.

Materials and Methods

1. Ovarian Collection: Feline ovaries were collected through the Jr. Surgery Program at the University of Illinois from intact and previously ovulated cats. Ovaries were either fixed in a buffered formaldehyde solution or frozen in liquid nitrogen or transported alive in 4C Phosphate Saline Solution (PSS) to the lab. Special thanks to Heather Soder for her support and assistance.

2. Histological Immunohistochemical Analysis: Fixed ovaries were sectioned at 7 μm and stained with Hematoxylin and Eosin or anti-αSMα for smooth muscle detection.

3. RNA Extraction and Endothelin Gene Expression: Eight frozen ovaries were homogenized by mortar and pestle and RNA was extracted using TRIzol (Thermo Fisher Scientific) followed by purification. RNA was used as a template for cDNA synthesis in a 1:1 ratio through reverse transcription. DNA was then used as template for detection and semi-quantitative PCR of genes involved in the Endothelin system.

4. Ovary Mounting: The buris was removed from each ovary under dissection microscope. The ovary was bisected longitudinally through a glass rod to separate a portion of the cortex and medulla away from the hilus and large ovarian arteries (Figure 1). The ovary was then mounted onto two 4mm pins of a myograph machine; the pins were punctured through the internal face of the sectioned ovary. Tissues were maintained under PSS buffer at 37°C with a continuous supply of oxygen and CO₂.

5. Tension Analysis: After equilibration, ovaries were stretched to a passive tension of 3mN. A wake-up protocol (6) was used to return muscle cells to an active state through intermittent washes with heated PSS buffer and a 60mM Potassium solution. ET-2 peptide was added in increments of 50nM, typically 25mM to determine the optimal solution concentration that would evoke a contractile response. Simultaneously, Tezosentan, which blocks both ET-A and ET-B receptors, was added step-wise by order of magnitude to generate an ideal dose to use for ET-2 antagonization, determined to be 140μM. To quantify feline contraction in response to these predetermined doses, a baseline tension measurement was first made under PSS buffer alone, and after the potassium solution was added. Next, the ovary was washed with a 200mM solution of ET-2 peptide in PSS and observed for 30 min. Lastly, tissues were maintained in a 140mM solution of the dual ET receptor antagonist Tezosentan and PSS buffer, and changes in tension were again measured. Twelve ovaries were used in tensile analysis (n=13).

Conclusion and Future Directions

- Tail-cuff Collection
- Ovarian Contraction
- Feline ET-2 Receptor System
- Ovarian Contraction Analysis

References