Effect of the oxytocin antagonist antocin and agonist decomoton on baboon luteal cell production and release of progesterone

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Objective: To evaluate the effect of oxytocin, its antagonist antocin, and agonist decomoton on baboon luteal cell P secretion.
Design: Prospective study.
Setting: Academic department of obstetrics and gynecology in a US medical school.
Animal(s): Luteal-phased timed corpora lutea (CL) from a cohort of adult cycling baboons *(Papio anubis).*
Intervention(s): Dispersed luteal cells from luteal phase baboon CL were cultured and treated with nothing (control), oxytocin (4–400 mU), antocin (4–400 μg), oxytocin with antocin, decomoton (4–400 μg), or oxytocin with decomoton.
Main Outcome Measure(s): Basal and hCG (10 U)-stimulated P were measured in the incubate.
Result(s): Basal P secretion did not change significantly with all peptides studied. The hCG-stimulated P secretion was significantly inhibited by oxytocin (4–400 mU), antocin (4–400 μg), and antocin combined with oxytocin, and with oxytocin, decomoton, and decomoton combined with oxytocin. Antocin (−12%) was less inhibitory than oxytocin (−25% to −22%). Antocin combined with oxytocin enhanced the inhibition to −35% to −39%. Decomoton had stronger (not significant) inhibition than oxytocin. Mean inhibition was 28%–35% with all doses of oxytocin, decomoton, or combined together.
Conclusion(s): Although decomoton, an oxytocin agonist inhibited hCG-stimulated luteal cell P secretion in vitro, antocin also acted like an agonist, rather than an antagonist, in inhibiting P secretion. (Fertil Steril® 2008;90:1366–71. ©2008 by American Society for Reproductive Medicine.)

Key Words: Oxytocin, antocin, decomoton, luteal cells, corpus luteum, oxytocin agonist, oxytocin antagonist, progesterone, luteolysis

It is well established that the corpus luteum (CL) of primates, such as the baboon (1, 2) the cynomolgus (3), and women (4–7), produces and secretes the neurohypophyseal peptide oxytocin. Furthermore, the peptide has been demonstrated to be bioactive (8). Oxytocin modulates luteal cell P production and secretion in vitro (1, 9) and pulsatile P secretion by the CL (10). In ruminants, oxytocin is luteolytic and immunization with oxytocin delays luteal regression and prolongs the estrus cycle (11). In sheep (12), goat (13–15), and cows, it has been suggested that the luteolytic contribution by oxytocin from the CL is mediated through increased production of endometrial prostaglandins (PG), which then act on the CLby the countercurrent vascular plexus between the uterus and the ovary (14, 16). In rats, oxytocin-induced luteolysis is mediated through uterine PGF_{2a} induced through oxytocin stimulation via uterine oxytocin receptors (17). In contrast, direct continuous luteal phase infusion of oxytocin into the CL of monkey induces luteolysis (18), indicating a more direct intraovarian or intraluteal action. In these preceding studies, the intervention was through manipulation of the oxytocin levels by either exogenous infusion or inhibition of endogenous oxytocin availability.

Given that oxytocin receptors have been demonstrated to be present in porcine (19) and primate CL (20), the action of oxytocin on luteal cells could be receptor-mediated within the CL itself. Therefore, we explored the role of oxytocin antagonist and agonist that could interfere with oxytocin action through its receptors. The oxytocin antagonist 1-deamino [D-Tyr (Et) 2, Thr4] OVT, antocin, is capable of inhibiting myometrial contractions (21) as well as inhibition of milk ejection in dairy cows (22) by blocking the oxytocin receptors in smooth muscle to the action of oxytocin. Not only is antocin antioxytocin, but it also has some antivasopressin 1_{α} receptor activity. On the other hand, decomoton demonstrates oxytocin agonist-like properties and competes for oxytocin receptor as an agonist. Thus, antocin and decomoton could be used to further evaluate the action of oxytocin on baboon luteal cell P production and secretion. Therefore, we conducted studies to examine the effects of oxytocin, its antagonist antocin alone, or in combination with oxytocin, and its agonist decomoton on baboon luteal cell P production and secretion in vitro.

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MATERIALS AND METHODS

Animals

Six adult, sexually mature, fertile, cycling female baboons (*Papio anubis*) with well-defined menstrual cycles were used for the study. The animals weighed 14–17 kg. The studies were approved by the Review Board for Animal Experimentation at the University of Illinois College of Medicine at Chicago and at the University of Texas Health Science Center at Houston, Texas, and were completed during our tenure at these two institutions. The studies were carried out in accordance with the principles and procedures described in the guide for the care and use of laboratory animals as approved by the National Institutes of Health (NIH 80-23). The baboons were maintained as previously described (1, 23). They had well-defined menstrual cycles and had been menstruating regularly for at least three cycles before being entered into the study. They were kept in individual cages in air-conditioned rooms and fed monkey chow ad libitum. Their menstrual cycles were monitored daily by the appearance and score of their perineal tumescence and serum P levels after deturgescence (1, 24, 25). The day of perineal deturgescence was equated to the day of LH surge. In the baboon, perineal deturgescence has been shown to be related to the LH surge by Koyama et al. (25), Stevens et al. (24), and our observations of more than 180 cycles (unpublished). Daily blood samples were drawn to determine the levels of plasma P and LH near the midcycle. Hence, each part of the luteal phase can be precisely timed. Luteectomy or ovariectomy was performed in three cycles in each animal and up to a maximum of six cycles or laparotomies in a lifetime for each animal. All animals had at least one rest cycle between the cycles during which operation was performed. We classified LH +1 to +5 as the early luteal phase, days LH +6 to +10 as the midluteal phase, and days LH +11 to +15 as the late phase.

Animal Surgery and Corpus Luteum Collection

Under general anesthesia induced with ketamine hydrochloride (10–12 mg/kg body weight) and maintained with endotracheal intubation and nitrous oxide-oxygen (1:1, vol/vol) with 3%–4% halothane as needed, a laparotomy was carried out through a lower abdomen midline incision. The reproductive structures were identified and examined for CL. Luteectomy was performed as previously described (1). The CL was immediately processed and used fresh for the studies described in this report. A total of 10 CL were used for the present study. The CL were individually processed and separately studied. Seven CL were from the midluteal phase (from day LH +7 through +10) and three were from late luteal phase (day LH+12 through +14). For the decomoton experiments, four midluteal phase CL (days LH +8 through +10) were used and for the antocin studies, three midluteal (days LH +7 through +9) and the three late luteal phase (days LH +12 through LH +14) CL were used.

Preparation of Dispersed Luteal Cell

Dispersed luteal cells were prepared by enzymatic cell disaggregation as previously described and established in our laboratory for baboon (1, 26) and human (6, 9) CL. Briefly, after luteectomy, the CL was placed in cold Ham's F-10 culture medium at 4°C and then microdissected to remove blood clots, unwanted connective tissue, and any nonluteal tissue. After weighing, a small cube was taken for histology, whereas the remaining tissue was minced with a pair of small scissors. The luteal cells were disaggregated and dispersed by incubating the minced tissue (100 mg tissue/1 mL of medium) in fresh Ham's F-10 containing 80 U collagenase, 100 μg DNAse, 150 μg trypsin inhibitor/mL streptomycin, and 0.25 μg fungizone (dispersion buffer). Cell dispersion was speeded up by flushing the minced tissues through Pasteur pipettes. The incubations were carried out in air. After a 10-minute interval the supernatant containing the dispersed cells was removed, the cells were centrifuged, and the pellet was taken up in Ham's F-10 culture medium. The remaining minced tissues were further treated with the dispersion buffer and after four to five repeat cycles, the pelleted cells were washed with media containing no enzymes. At the end of the enzymatic digestion, the cell suspension was filtered through nylon mesh of 105 μm twice and the filtrate was centrifuged at 4°C for 10 minutes at $100 \times g$. The cells in the pellet were suspended in the medium. After determination of the cell number by counting on a hemocytometer, the volume of the suspension was adjusted with medium to obtain a ratio of 100,000 cells per 100-μL suspension.

Aliquots of this cell suspension were individually placed into separate wells of a sterile multiwell disposable plate for the experiments. Incubations were performed in sextuplicates for each experimental point in a shaking water bath at 37°C in air. At the end of 3 hours of incubation for each experiment, the reactions were terminated by freezing the incubate in a −70°C freezer and stored as such until ready for P measurement. Viability of the dispersed luteal cells for each batch of preparation was determined by trypan blue dye uptake after cell counting and also at the end of each experimental batch. For the present set of studies with antocin and decomoton, the cell viability was consistently more than 85% of the dispersed cells and the percentage of viable cells remained similar at the end of the 3 hours of incubation for the experiments.

Antocin and Decomoton

Antocin and decomoton were generous gifts from Dr. Per Melin of Ferring Pharmaceuticals in Finland and Dr. Daniel M. Linkie of Ferring Laboratories, Inc, Suffern, New York. Antocin (CAP 428) had 99.1% purity and bioactivity on rat uterus in vitro with a pH 2 = 8.4 + 0.1 (antagonism), whereas decomoton (batch 296) had a purity of 97.7% and a bioactivity of 8.8 IU/mg (80.9–124) (agonism). Both substances were readily dissolved in saline.

Experimental Design for In Vitro Studies

As shown in our previous studies (1, 9) optimal conditions were obtained using 50,000 cells per incubate in 1 mL for 3

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