Drugs That Block Smooth Muscle Contraction Inhibit In Vivo Ovulation in Hamsters

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ABSTRACT We have demonstrated previously that smooth muscle cells (SMC) in the base of hamster follicles contract minutes before ovulation. The contraction of these cells correlates well in time with the development of a constriction in the base of the follicle. The purpose of this study was to determine whether contraction of SMC (1) produces this constriction and (2) is necessary for ovulation. We treated prevulatory hamster ovaries with six classes of drugs (lanthanides, calcium antagonists, local anesthetics, prostaglandins, cAMP modulators, and cytochalasin B) known to inhibit SMC contraction in other tissues. Drugs were injected into the experimental bursal cavity of a hamster 3 hours before ovulation; the contralateral ovary received no treatment and served as a control. Three hours after ovulation, the number of ruptured follicles on experimental and control ovaries were compared by light and electron microscopy. All the smooth muscle (SM) inhibitors, except those that affect cAMP levels and cytochalasin B, prevented (1) contraction of follicular SM, (2) constriction of the base of the follicle, and (3) ovulation. These results support the idea that contraction of follicular SMC constricts the follicle and is required for ovulation. The results further show that follicular SMC are activated by an influx of extracellular calcium and that prostaglandin F₂α may be involved in promoting this contraction.

Smooth muscle cells (SMC) have been identified in the wall of mature follicles of every mammalian ovary examined by transmission electron microscopy; however, their role in ovulation is not clear. In hamsters, we have shown that SMC, which are most abundant in the theca externa at the base of the follicle, contract during the final minutes before rupture of the follicle (Martin and Talbot, '81). At the time these cells contract, a V-shaped constriction forms in the basal follicle wall, and this gradually eliminates the basal part of the antrum. We have suggested that contraction of follicular SMC produces this constriction, which in turn (1) squeezes the cumulus mass apically and facilitates its escape from the antrum, and (2) increases tension in the enzymatically weakened apical follicle wall, thereby causing its final distention and eventual rupture. Further contraction of these SMC may also aid in collapse of the follicle wall after rupture.

In this paper, we address the question of whether or not the contraction of SMC is required for (1) constriction of the base of the follicle, and (2) ovulation. We have treated hamster ovaries in vivo with 17 drugs known to inhibit the contraction of smooth muscle in other systems and have assessed their effect on ovulation. Thirteen of these drugs prevented contraction of follicular SM, constriction of the follicle, and ovulation. These experiments provide information on the physiology of follicular SM and add further support to the idea that contraction of these cells is necessary for the rupture of ovarian follicles in hamsters.

MATERIALS AND METHODS

Sexually mature female golden hamsters (Mesocricetus auratus), 8–20 weeks old (90–140 gm), were used throughout this study. Maintenance of the animals and the hormonal control of the timing of ovulation have been described previously (Martin et al., '81).

The effect on ovulation of various drugs that inhibit SM contraction was assessed using an in vivo technique described previously (Martin et al., '81). In the present study, 25 μl of a test solution, containing a drug dissolved in normal

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saline, were injected into the bursal cavity surrounding the experimental ovary about 3 hours before expected ovulation. The contralateral ovary received no treatment and served as a control. Two hours after expected ovulation, the ovaries were removed, and the number of ruptured follicles on experimental and control ovaries were counted. Both ovaries from each hamster were processed and examined by light and transmission electron microscopy (TEM) as described previously (Martin and Talbot, '81).

These are the 17 chemicals we tested: (1) lanthanides (with the exception of LaNO₃, these were provided by Dr. White, Department of Biochemistry, University of California, Riverside): lanthanum nitrate (Sigma), europium nitrate, gadolinium nitrate, praseodymium nitrate, and others.

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**Fig. 1.** The effect of (A) LaNO₃ and (B) other lanthanides on ovulation. The number of follicles that ruptured on the experimental ovary is expressed as a percentage of the number of follicles that ruptured on the control ovary. The latter is 100%. The number of hamsters used for each concentration of LaNO₃ is as follows: 1 mM (n = 9); 5 mM (n = 22); 10 mM (n = 17); 20 mM (n = 9). In B, five hamsters were tested for each of the following lanthanides: Eu, Europium; Gd, Gadolinium; Pr, Praseodymium; Yb, Ytterbium; and Ce, Cerium.

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**Fig. 2.** The effect of two calcium antagonists on ovulation. The data is expressed as in Figure 1. The number of hamsters used for the following concentrations of Verapamil were: 1 mM (n = 4); 10 mM (n = 6); 25 mM (n = 5); and 50 mM (n = 5). For Diltiazem: 25 mM (n = 4); and 50 mM (n = 6).
trate, ytterbium nitrate and cerium oxide; (2) calcium antagonists: diltiazem hydrochloride (Dr. R. K. Browne, Marion Labs, MI) and verapamil hydrochloride (isoptin, Dr. E. B. Kirsten, Knoll Pharmaceutical Co., NJ); (3) local anesthetics: dibucaine hydrochloride (nupecaine, Dr. C. A. Brownley, Jr., Ciba Geigy, NJ) and lidocaine hydrochloride (xylocaine, Dr. H. G. Vassallo, Astra Pharmaceutical Products Inc., MA); (4) prostaglandins (PG), PG inhibitors, and PG antagonists: indomethacin (in-

docin, Merck, Sharp and Dohme, PA), PGE (Sigma), and flufenamate (Dr. R. D. Westland, Warner-Lambert Co., MI); (5) cAMP modulators: papaverine hydrochloride (Sigma), theophylline (Sigma), and N6, O12'-dibutyryl adenosine 3',5'-cyclic monophosphoric acid (db-cAMP, Sigma Grade II); and (6) cytochalasin B (Sigma).

We injected drugs into the bursal cavity at concentrations higher than those generally used to inhibit SM contraction in other sys-

![Graph A: Papaverine Concentration vs. Percent Ovulation](image)

**Fig. 3.** The effect of three drugs that alter the intracellular concentration of cAMP on ovulation is expressed as explained in Figure 1. The following number of hamsters were tested for each drug: Papaverine: 5 mM (n = 6); 10 mM (n = 4); 20 mM (n = 7); and 50 mM (n = 4). Theophylline: 10 mM (n = 4); and 1 mM (n = 3). db-cAMP: 10 mM (n = 4); 1 mM (n = 4); and 0.1 mM (n = 4).

![Graph B: Theophylline Concentration vs. Percent Ovulation](image)

![Graph C: db-cAMP Concentration vs. Percent Ovulation](image)

![Graph D: Dibucaine Concentration vs. Percent Ovulation](image)

**Fig. 4.** The effect of two local anesthetics is presented as described in Figure 1. The number of animals tested is as follows: (A) Dibucaine, 1 mM (n = 3); 10 mM (n = 3); 20 mM (n = 7); and 40 mM (n = 4); and (B) Lidocaine, 10 mM (n = 2); and 20 mM (n = 3).
tems. This is because solutions are diluted by bursal cavity fluid upon injection and drugs are gradually cleared from the bursal cavity with time (Martin et al., ’81). Thus, the effective drug concentration at the site of ovulation is similar to concentrations used in other studies.

RESULTS

Effect of drugs on ovulation

Figures 1–5 show the effects of various inhibitors of SM contraction on in vivo ovulation. For each hamster, the number of follicles that rupture on the control (untreated) ovary was considered as 100% ovulation; the number of follicles that ruptured on the experimental ovary is expressed as a percentage of the value for the control ovary. All drugs, with the exception of cytochalasin B and those that alter cAMP concentration, reduced the number of follicles ovulating on the experimental ovary in a dose-dependent manner. All mature follicles on control ovaries ruptured; in the rare instances when ovulation did not occur on the control ovary, the animals were eliminated from the study. Although cytochalasin B would not be expected to inhibit SM contraction, it was tested (Fig. 6) because it has been shown to block ovulation in lower vertebrates (Schroeder, ’73).

Morphology of treated follicles

Follicles on control and experimental ovaries were fixed 2–3 hours after expected ovulation and examined by light and electron microscopy. Ruptured follicles from both control and experimental ovaries appeared similar (Fig. 7A). Thick sections through these follicles revealed that the antrum had been nearly eliminated by the close apposition of folds of the follicle wall (Fig. 8A). The theca externa in the basal hemisphere of follicles remained hemicircular and did not parallel the convolutions of the inner layers of the follicle wall. SMC in this layer (Fig. 9A) had the features of contracted cells, including a highly indented plasma membrane (Martin and Talbot, ’81).

![Diagram](image)

Fig. 5. The effect on ovulation of three drugs that affect the synthesis and metabolism of prostaglandins. The data is presented as described in Figure 1. The following number of hamsters were tested: (A) Indomethacin, 0.7 mg/ml (n = 7), 1.4 mg/ml (n = 9); (B) PGE₃, 0.1 μg/ml (n = 3), 1 μg/ml (n = 5), 10 μg/ml (n = 6), 20 μg/ml (n = 6); (C) Flufenamate, 20 mM (n = 6); and 50 mM (n = 7).

![Diagram](image)

Fig. 6. The effect of cytochalasin B on ovulation as described in Figure 1. Six hamsters were tested at each concentration.
Fig. 7. Photographs of follicles at 3 hours after expected ovulation. The follicle in A is typical of follicles on control ovaries. The follicle in B was inhibited from rupturing by in vivo treatment with 10 mM LaNO₃.

Fig. 8. Photographs of thick sections through follicles similar to those shown in Figure 7. The ruptured follicle in A is typical of follicles from the control ovary, whereas the follicle in B was inhibited from rupturing by 0.7 mg/ml Indomethacin.

In contrast, unruptured follicles from experimental ovaries (1) were spherical and protruded about 1 mm from the surface of the ovary (Fig. 7B); (2) had thick apical follicle walls; (3) showed no signs of constriction (Fig. 8B); and (4) possessed relaxed SMC (Fig. 9B) with smooth plasma membranes and nuclear envelopes. Similar features are characteristic of unruptured follicles two hours before ovulation (Martin and Talbot, '81). The only exceptions to these observations occurred in one out of eight follicles treated with 0.1 μg PGE/ml and in four out of 12 follicles treated with 0.2 mM indomethacin. These follicles had slightly V-shaped walls but the apical wall remained thick and the SMC appeared relaxed. Cytochalasin B at a concentration of 100 μg/ml inhibited ovulation, but the experimental
Fig. 9. Transmission electron micrographs of SMC at the base of follicles in which ovulation occurred normally (A), and in which rupture was prevented by treatment with SM inhibitor (B). In A, the plasma membrane is highly convoluted—a typical feature of contracted SMC. In B, the ovary was treated with 10 μg/ml PGE and the plasma membrane and nuclear envelope are smooth—that is, characteristic of the uncontracted state. × 27,500.
ovary was abnormal—that is, markedly redden—and the antra of mature follicles contained large blood clots.

**DISCUSSION**

We have shown that when drugs known to inhibit SMC contraction in other systems are injected into the bursal cavities of hamsters 3 hours before expected ovulation the following events do not occur: (1) contraction of follicular SMC; (2) constriction of the base of the follicle; and (3) ovulation. From this, we conclude that contraction of SMC causes the base of the follicle to become constricted and that the development of this constriction is probably necessary for ovulation. The latter point must still be qualified because the drugs we tested may have affected processes in addition to SM contraction. In order to lessen the likelihood of this, we treated ovaries only during the final 2–3 hours before expected ovulation, when most preovulatory events are complete. Nevertheless, these events do occur, at least in part during this time—inflammatory responses (Espey, '80) and weakening of the follicle wall by hydrolytic enzymes (Strickland and Beers, '79)—may be affected by several of the drugs we tested. The literature on the effect of drugs on these processes includes contradictory information such that some studies report that indomethacin and the lanthanides increase inflammation and proteolytic activity (Houck et al., '68; Brown and Pollock, '70b; Dayer et al., '76; Triggie and Triggie, '76; whereas other reports claim they inhibit these processes (Brown and Pollock, '70a; Suzuki et al., '76; Web et al., '77). We also tried to minimize the probability that the inhibition of ovulation was due to processes other than SM contraction by using six different classes of SM inhibitors.

Our results provide information on the physiology of follicular SMC. In general, SMC may be divided into two types, based on whether initiation of contraction requires calcium from an extracellular or intracellular store (Somlyo and Somlyo, '75). This activator calcium is released upon stimulation of the cell and results in the interaction of myofilaments. The two types of SMC may be distinguished if the tissue can be placed in calcium-free media and contraction stimulated. Although this has not yet been possible with the hamster follicle, two lines of evidence suggest that follicular SMC utilize external stores of activator calcium. First, SMC that utilize internal calcium typically have cisternae of sarcoplasmatic reticulum in close association with the plasma membrane (Devine et al., '72; Somlyo and Somlyo, '76), whereas those requiring external calcium do not. Follicular SMC belong in the latter category. Second, all of the drugs that inhibited ovulation, lanthanides, calcium antagonists, local anesthetics and PG, are known to block calcium influx (Feinstein, '64; Van Bree, '69; Ainsworth et al., '79; Martin and Richardson, '79; Fleckenstein, '77). The drugs we used that alter the intracellular concentration of calcium, db-cAMP, papaverine, and theophylline (Andersson et al., '75; Demesy-Waedele and Stocklet, '75; Kubovetz et al., '76), had no significant effect on ovulation. It is important to note that rabbit ovaries treated with EGTA had fewer ovulating follicles than untreated controls (Wallach et al., '78). While the role of SMC in rabbit ovulation is still ambiguous (Espey, '78) and chelation of extracellular Ca²⁺ could alter several other processes associated with ovulation, the observations of Wallach et al. ('78) are consistent with the idea that blocking influx of extracellular Ca²⁺ into SM of rabbit follicles inhibits their contraction and subsequent ovulation.

Based on experiments presented in this paper, follicular SMC require an influx of calcium to initiate contraction, but what triggers this event? In general, SMC may be stimulated by (1) hormones, (2) nerves, and (3) local changes in the muscle itself. Although it is not known what mechanisms operate in follicular SMC, the effects of hormones and nerves on ovarian contractility have recently been reviewed (Owman et al., '79), and the following evidence has accumulated to support the idea that PGs may be involved. In ovaries, the concentrations of both PGE and PGF₂α increase in preovulatory follicles (LeMaire et al., '73; Yang et al., '73), and drugs, like indomethacin, that block this rise inhibit ovulation (Lau et al., '74; Hamada et al., '77). Although the precise role of these PGs in ovulation needs further clarification, they have been shown to affect follicle contractility. In earlier studies, it was demonstrated that PGE inhibits ovarian contractions and ovulation (Hamada et al., '77; Lerner et al., '78). The present report agrees with these
observations and adds to them by showing that PGE inhibits contraction of follicular SMC in hamsters. Its mode of action on ovarian SMC is not presently understood.

Because PGF<sub>2α</sub> increases the contractility of follicular strips from the rabbit and human ovary, several investigators have suggested that the preovulatory rise in ovarian PGF<sub>2α</sub> is responsible for stimulating the contraction of follicular SMC. Our observations with flufenamate and indomethacin support these earlier studies. Flufenamate was tested because it was previously shown to inhibit PGF<sub>2α</sub>-induced contraction of lung SMC, but not to effect that SMC response to PGE (Collier and Sweatman, '68). Thus, the inhibition of contraction of hamster follicular SMC by flufenamate is consistent with the suggestion that this contraction is modulated by PGF<sub>2α</sub>. Indomethacin inhibits synthesis of both PGE and PGF<sub>2α</sub>. Because we have shown that PGE inhibits contraction of hamster follicular SM, it is reasonable to suggest indomethacin was effective because it decreased PGE<sub>2α</sub> synthesis. Presumably the contraction of SMC is affected either by changes in the proportion of PGE<sub>2α</sub> to PGE (Yang et al., '73), or by differences in the distribution of these two prostaglandins within the follicle.

Local changes in SMC themselves may also lead to contraction. We suggest that local changes in the follicular SMC itself may be important in stimulating PG synthesis and release which could lead to SMC contraction. During maturation of the follicle the antrum swells and this results in thinning of the apical follicle wall and stretching of cells throughout the entire follicle. SMC surrounding many fluid-filled structures like the bladder, stomach, and blood vessels, respond to stretch by contracting (Kosterlitz and Watt, '75). Stretching of SMC from the uterus and antrum of the dog stomach promotes the release of PGE<sub>2α</sub>, which causes contraction of these SM (Piper and Vane, '71). It has been suggested that the release of the PG and the resulting contraction is a cellular defense mechanism to prevent disruption of the cell membrane and possible rupture of the cell (Piper and Vane, '71). If such a system is operating in follicular SMC, a localized elevation of PG may develop around the SMC in addition to the general rise in PG noted in the entire follicle. Either of these pools of PG may be involved in initiating follicular SM contraction.

In conclusion, we have shown that several classes of drugs that inhibit SMC contraction prevent (1) contraction of follicular SMC, (2) constriction of the basal follicle wall, and (3) ovulation. These results add further support to the idea that the contraction of SMC is involved in the rupture of hamster follicles. Moreover, the contraction of follicular SMC requires an influx of calcium and most likely a specific concentration of PG in the surrounding medium.

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LITERATURE CITED


