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INFLUENCE OF ESTROGEN AND PROGESTERONE TREATMENT ON OVARIAN CONTRACTILITY IN THE MONKEY*

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The presence of smooth muscle and autonomic nerve fibers in the ovary[1,2] and the ability of ovarian tissue to contract[3-9] have led to the suggestion that ovarian contractility plays a significant role in the mechanism of ovulation. Ovarian contractions have been clearly demonstrated both in vitro and in vivo in the cat, rabbit, monkey, and human.[3-9] Uterine and oviductal smooth muscle have been extensively investigated; their contractile characteristics have been shown to vary, depending on hormonal status (eg, during different phases of the menstrual cycle, throughout pregnancy, or when under the influence of exogenous estrogens and progesterone).[10-15]

Previously, we reported that contractions in rhesus monkey ovaries were more prominent during the follicular phase of the menstrual cycle. Monkey ovarian contractile responsiveness to catecholamines and prostaglandins was also enhanced during the preovulatory phase, suggesting that local concentrations of estrogens and progesterone might influence ovarian contractility.[6] The present investigation was designed to study contractility of the monkey ovary in vitro and its responses to catecholamines, prostaglandins, and cholinergic drugs after treatment with relatively high doses of estrogens or progesterone.

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MATERIALS AND METHODS

Five female rhesus monkeys (*Macaca mulatta*) weighing 5 to 6 kg each were used in this experiment. The monkeys were housed individually, fed a diet of Purina monkey chow and fresh fruit, and had access to water ad libitum. Room temperature was kept constant at 70 F. Regular lighting was maintained with 14 hours of light and 10 hours of darkness. Vaginal smears were taken daily before and during the experiment to detect vaginal bleeding. The cycle immediately preceding the experiment was 26 to 30 days in length. Treatment was started with conjugated estrogens, (5 mg/day I.M., Premarin, Garfield Davies, Ayerst Labs, Inc, NY) on the first day of the menstrual cycle and continued for 21 days. At the conclusion of this three-week interval of estrogen administration, a unilateral oophorectomy was performed. The following day a second treatment was begun using progesterone in sesame oil (25 mg/day I.M.) for 21 days. At the end of the three-week course of progesterone administration, a second laparotomy was performed and the

TABLE 1.—*Drugs and Dosages*

Drug	Dose (μg/ml)	Molarity
Norepinephrine bitartrate	0.08 - 1.0	1.7×10^{-7} - 2.1×10^{-6}
Phenoxybenzamine HCL	20 - 40	5.9×10^{-5} - 1.2×10^{-4}
Isoproterenol HCL	0.4 - 2.0	1.6×10^{-6} - 8.1×10^{-6}
Propranolol HCL	0.6 - 1.6	2.0×10^{-6} - 5.4×10^{-6}
Prostaglandin $F_2\alpha$	4.0	1.1×10^{-5}
Prostaglandin E_2	4.0	1.1×10^{-5}
Atropine sulfate	1.0 - 2.0	2.6×10^{-6} - 5.2×10^{-6}
Neostigmine methyl sulfate	2.0	4.5×10^{-6}
Pilocarpine nitrate	8.0	3.0×10^{-5}

uterus and remaining ovary were removed.

Laparotomies were performed under phencyclidine hydrochloride anesthesia (10 mg I.M., Sernylan, Bio-ceutic Laboratories, Inc, St. Joseph, Mo). After each oophorectomy, the ovary was immediately placed in a muscle chamber containing oxygenated Krebs-Ringer solution at 37° C. The ovary was then attached by 6-0 black silk to a Statham Universal transducer. The transducer was connected to a Beckman RM dynograph and contractions were recorded as previously described.[4-6]

All ten ovaries were small (between 5 and 8 mm in length) and lacked pronounced follicular activity or recent ovulation points. In selected cases, the ovary was bisected and one half of the specimen was prepared for histologic studies. After hysterectomy, the endometrium was fixed in Bouin's solution for histologic examination.

Adrenergic agents (norepinephrine, phenoxybenzamine, isoproterenol, and propranolol), prostaglandins $F_2\alpha$ and E_2 ($PGF_2\alpha$ and PGE_2), and cholinergic drugs (atropine, pilocarpine, and neostigmine) were added to the solution in the doses listed in Table 1. Ovarian contractile patterns and responses to pharmacologic agents following estrogen treatment were compared to those following progesterone administration.

RESULTS

Estrogen-treated group. This group was composed of two right and three left ovaries (Table 2). Only one ovary demonstrated rhythmic contractions; in this case spontaneous, irregular contractions were recorded during the first 30 minutes of the experiment and became rhythmic following the addition of norepinephrine to the bath solution (Fig. 1A). A second ovary demonstrated spontaneous, irregular contractions. Contractions were not detected in the remaining three ovaries, nor was any response to the

TABLE 2.—*Contractility of Five Monkey Ovaries After Estrogen and Progesterone Treatment*

	Estrogen				Progesterone			
		No. of ovaries responding[a]				No. of ovaries responding[a]		
Drug	No. treated	Frequency	Amplitude	Tone	No. treated	Frequency	Amplitude	Tone
Norepinephrine	5	+1	+2	+1	5	+2	+3	+1
Phenoxybenzamine	2	−2	−2	0	4	−2	−2	−2
Propranolol	4	0	0	0	4	0[b]	−1,+1	0
Isoproterenol	3	0	0	0	4	−1	0	−2
Prostaglandin $F_2\alpha$	5	0	+2	+1	5	+3	+4	+3
Prostaglandin E_2	3	−1	0	0	4	−1	−3	−3
Neostigmine	4	−1	−1	−1	3	+1	+1	0
Pilocarpine	3	−1	−1	−2	2	0	0	+2
Atropine	5	0	0	0	5	0[b]	−1	−1

[a] + = increase, − = decrease in parameter cited.

[b] Three ovaries used.

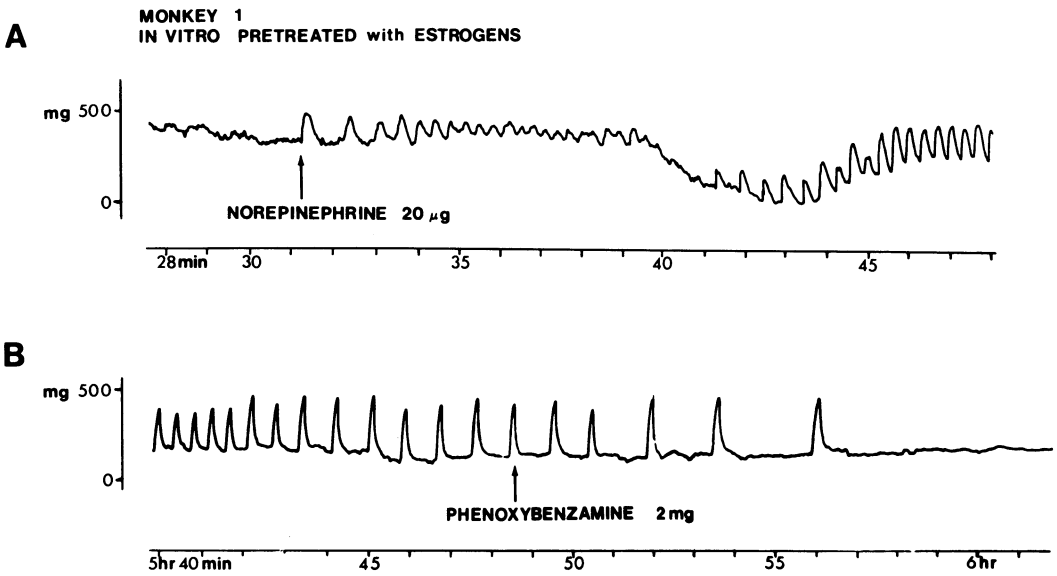

FIG. 1. In vitro recordings of ovarian contractions. Ovary removed from monkey 1, pretreated with estrogens. (*A*). Conversion of an irregular contractile pattern to a regular pattern after addition of norephinephrine (20 μg). (*B*). Cessation of contractions after addition of phenoxybenzamine (2 mg).

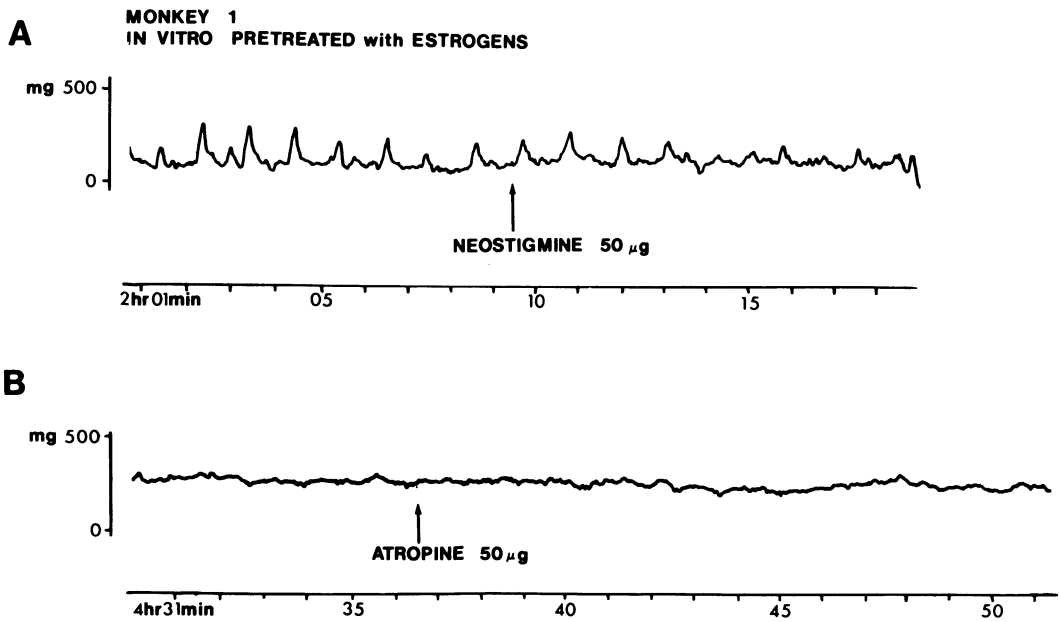

FIG. 2. In vitro recordings of ovarian contractions. Ovary removed from monkey 1, pretreated with estrogens. (*A*). Decrease in contractile frequency and amplitude after addition of neostigmine (50 μg). (*B*). No change in contractile patterns after addition of atropine (50 μg).

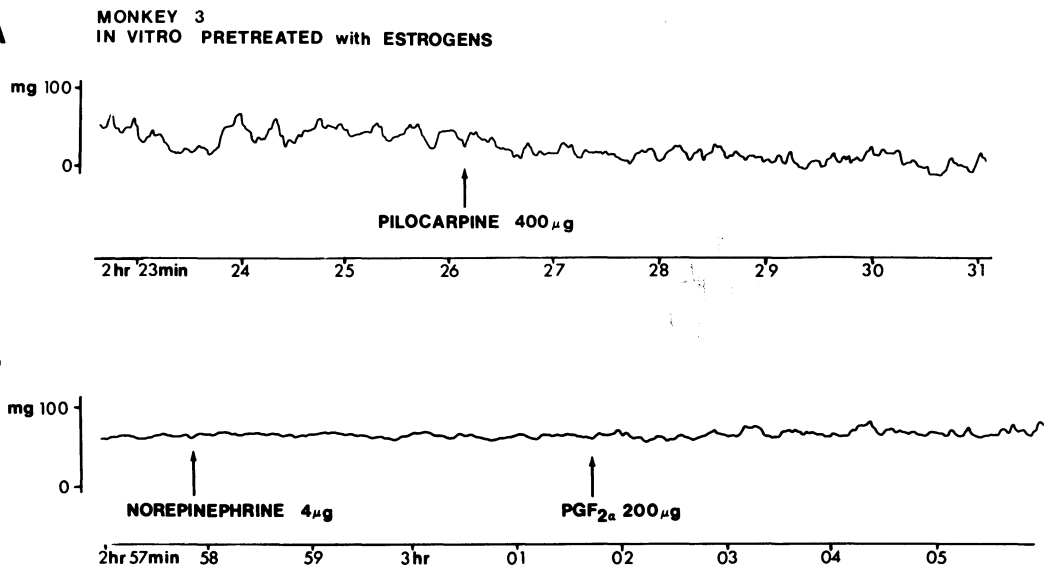

FIG. 3. In vitro recordings of ovarian contractions. Ovary removed from monkey 3, pretreated with estrogens. (*A*). Decrease in contractile amplitude and tone after addition of pilocarpine (400 μg). (*B*). After a change of solution after pilocarpine, addition of norepinephrine (4 μg) failed to reinstate contractions; $PGF_2\alpha$ (200 μg) was followed by a resumption of contractile pattern.

various pharmacologic agents observed. In only a few instances were responses to catecholamines or prostaglandins noted. In each case the response was consistent with previous observations,[4-6] namely enhanced ovarian contractility following norepinephrine and $PGF_2\alpha$ and inhibition after phenoxybenzamine and PGE_2 (Fig. 1B). This response was apparent in at least one of the three parameters studied (tone, frequency, amplitude). No responses were observed after the addition of propranolol or isoproterenol. Cholinergic agonists exhibited an inhibitory action while atropine had no apparent effect on ovarian contractility (Fig. 2). Pilocarpine decreased frequency, amplitude, and tone in one case in which contractility had been previously stimulated with $PGF_2\alpha$. However, increased tone and decreased amplitude were observed in the same preparation following pilocarpine when norepinephrine had been added previously. This negative effect of pilocarpine could not be overcome by norepinephrine or $PGF_2\alpha$. Even when the bath solution was changed, ovarian contractions failed to return spontaneously or in response to norepinephrine; however, contractions did resume after addition of $PGF_2\alpha$ (Fig. 3). In another case, $PGF_2\alpha$ increased frequency and amplitude of contractions previously decreased by neostigmine.

Progesterone-treated group. This group included three right and two left ovaries (Table 2). Four ovaries displayed spontaneous, irregular contractions, and one ovary displayed spontaneous rhythmic contractions, starting 18 minutes after the experiment began (Fig. 4A). Of the four ovaries which demonstrated irregular contractility, contractions became rhythmic in one in response to $PGF_2\alpha$ 40 minutes after the ovary had been placed in the muscle chamber. In another, norepinephrine induced transitory rhythmic contractions 25 minutes after the recording was started (Fig. 4B). In this case $PGF_2\alpha$ reinstated regular contractions of greater amplitude when added three hours later (Fig. 4C). Rhythmic contractions could not be detected in the other two ovaries at any time.

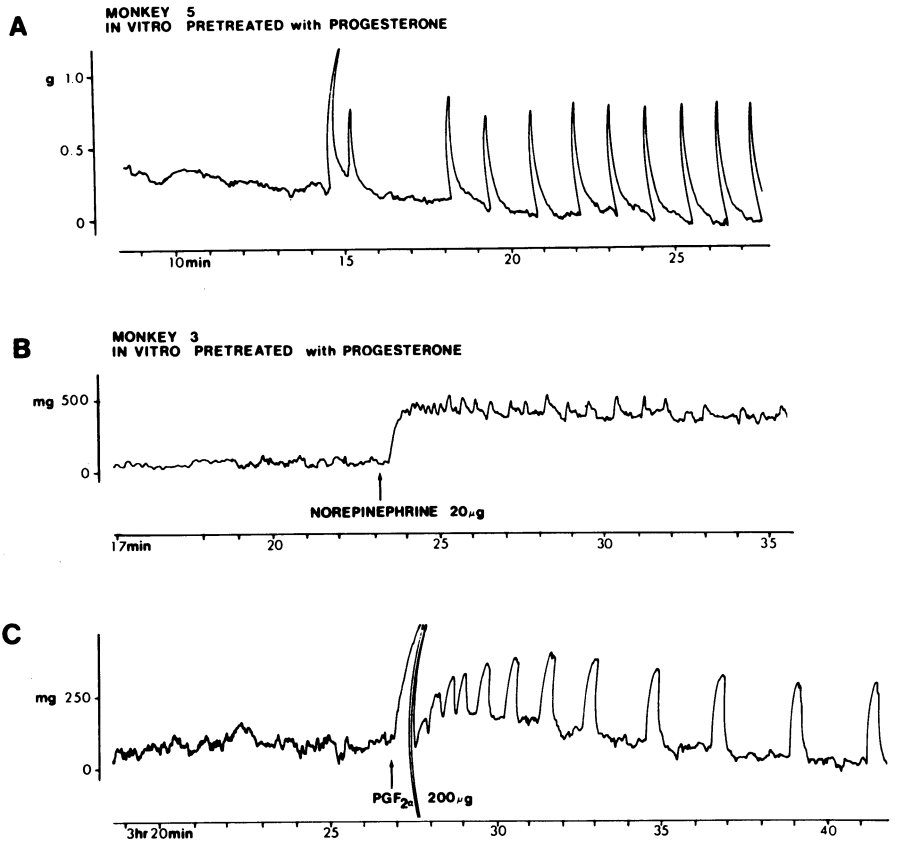

FIG. 4. In vitro recordings of ovarian contractions. (*A*). Ovary removed from monkey 5 after progesterone treatment showing spontaneous regular contractions. (*B*). Ovary removed from monkey 3 pretreated with progesterone, showing initiation of rhythmic contractions after addition of norephinephrine (20 μg). (*C*). High amplitude regular contractions after addition of $PGF_2\alpha$ (200 μg).

Responses to prostaglandins were more prominent in this group than in the ovaries studied after estrogen treatment. $PGF_2\alpha$ led to an increase in frequency, amplitude, and tone in three cases (Fig. 5A). Amplitude was increased in one ovary which demonstrated irregular contractions; another ovary with irregular contractions exhibited no response to $PGF_2\alpha$. Prostaglandin E_2 led to a decreased frequency, amplitude, and tone in one ovary with rhythmic contractions, and, in one instance, led to a temporary cessation of contractions (Fig. 5B). In two other cases, both amplitude and tone were decreased following PGE_2. The only ovary which did not respond to PGE_2 also failed to respond to phenoxybenzamine which was added later in the experiment.

Of the three ovaries which displayed rhythmic contractions, norepinephrine led to increased frequency, amplitude, and tone in one, increased frequency and amplitude in another, and increased amplitude only in the third. Neither of the two ovaries with irregular contractions responded to norepinephrine. Phenoxybenzamine decreased frequency, amplitude, and tone in two ovaries with rhythmic contractions and completely stopped contractions in another (Fig. 6). This alpha-adrenergic blocker failed to affect either the ovary with rhythmic contractions or the two ovaries with irregular contractions. As anticipated, all three responses

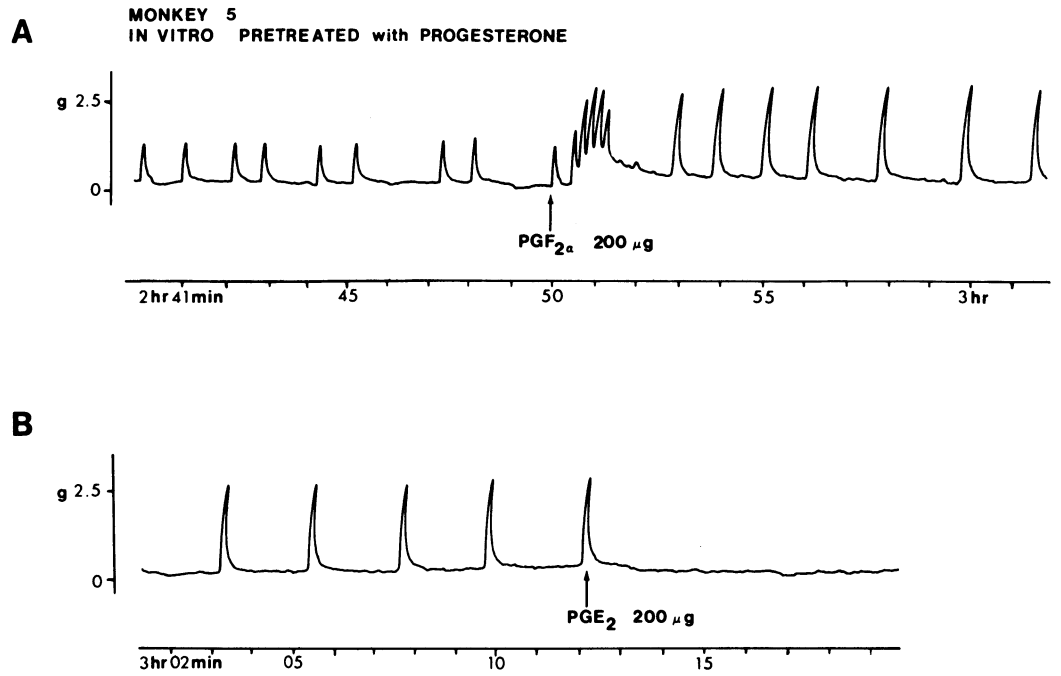

FIG. 5. In vitro recordings of ovarian contractions. Ovary removed from monkey 5 after progesterone treatment. (*A*). Increase in amplitude after addition of $PGF_2\alpha$ (200 μg). (*B*). Cessation of contractions after addition of PGE_2 (200 μg).

observed following isoproterenol were inhibitory. Propranolol slightly increased amplitude in one case and decreased it in the other.

Of significant interest was the response to cholinergic drugs in this group. Pilocarpine or neostigmine increased at least one of the three contractile parameters studied (Fig. 7.) This observation was the opposite of that in the estrogen-treated group. Atropine decreased amplitude and tone in one case, and blocked the stimulatory effect of pilocarpine in the other.

Histology. No significant differences were noted in ovarian histology between the two groups. In general, the ovaries demonstrated capsular thickening, nu-

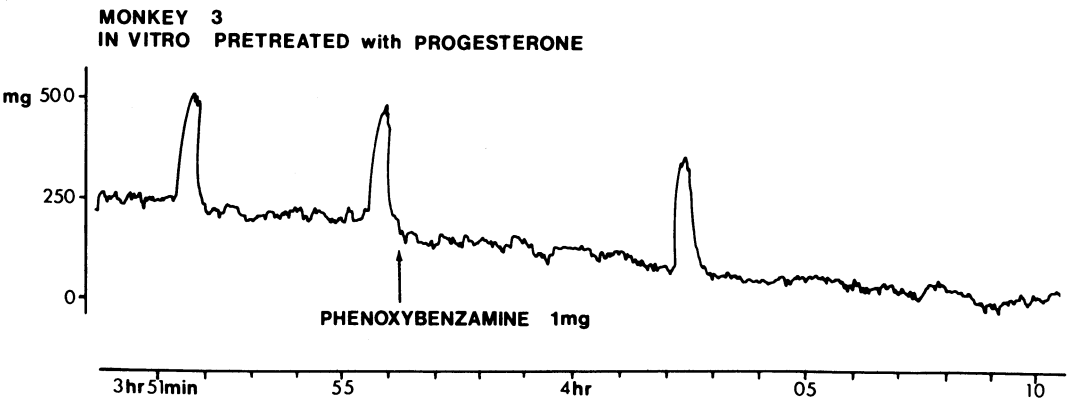

FIG. 6. In vitro recordings of ovarian contractions. Ovary removed from monkey 3 after progesterone treatment, showing a cessation of contractions after addition of phenoxybenzamine (1 mg).

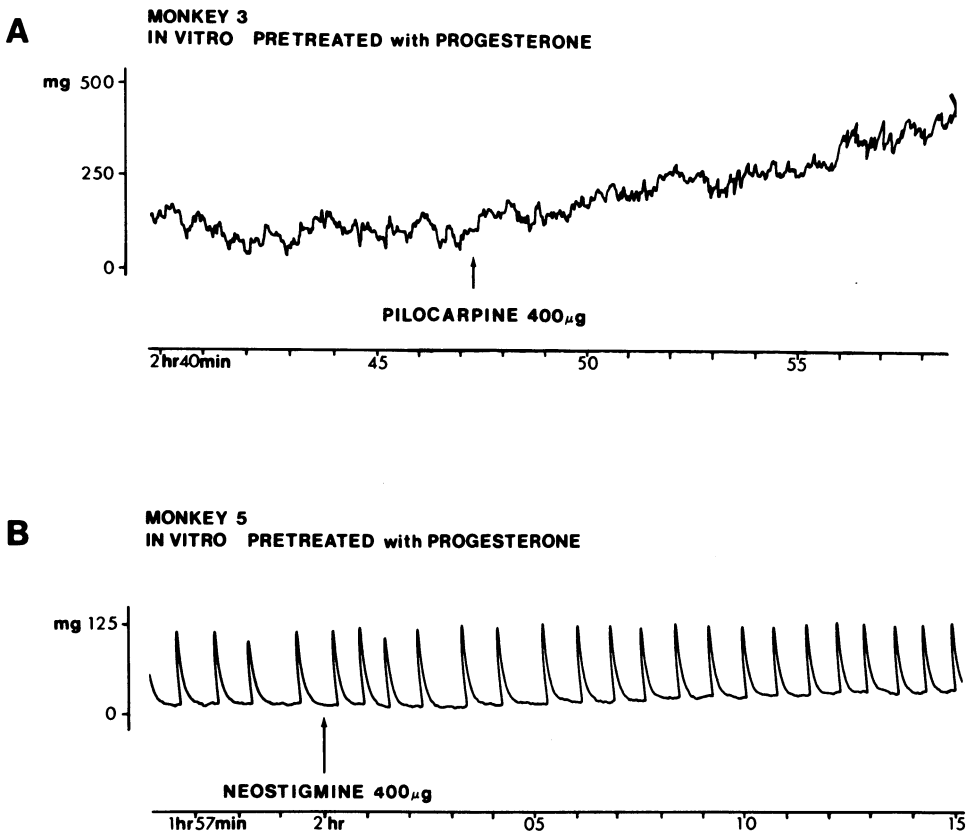

FIG. 7. In vitro recordings of ovarian contractions. (*A*). Ovary removed from monkey 3 after progesterone treatment showing increased tone after addition of pilocarpine (400 μg). (*B*). Ovary removed from monkey 5 after progesterone treatment showing a slight increase in contractile frequency after addition of neostigmine (400 μg).

merous small subcapsular cystic follicles, and deeper follicles in various early stages of development. With the Gomori trichrome stain for smooth muscle tissue, varying concentrations of smooth muscle fibers were demonstrated in the stroma, especially close to the follicles. No correlation could be drawn between the degree or pattern of ovarian contractility and the quantity of muscle tissue observed in any instance. Some of the ovaries which failed to contract had a generous distribution of smooth muscle fibers. The endometrium in each of the five cases was thick and showed numerous tortuous glands with intraluminal inspissated secretion. The stroma was edematous and clearly demonstrated a predecidual reaction. In none of the monkeys was breakthrough bleeding observed during the course of either estrogen or progesterone treatment.

DISCUSSION

Ovarian steroids have been shown to influence the spontaneous activity and pharmacologic sensitivity of the human uterus.[10-14] Likewise, oviductal motility is known to be influenced by estrogen and progesterone.[15] The relationship of these hormones to ovarian smooth muscle contractility, however, is not well defined. Ovarian contractility has been reported to be more prominent during the preovulatory phase of the

menstrual cycle than following ovulation in the rhesus monkey.[6] Coutinho and Maia[8] described greater activity in human ovaries studied in vivo immediately following administration of human menopausal gonadotropin or human chorionic gonadotropin.

In the present study we were able to demonstrate contractions in monkey ovaries, with pronounced rhythmicity in certain instances, especially after treatment with progesterone. The exact physiologic role of ovarian contractions has not been established, but recent experience has related ovarian contractility to the process of ovulation. Adrenergic drugs and prostaglandins are known to influence ovulation in different species.[16-18] These drugs also affect ovarian contractility.[4-6] It is reasonable to suggest that local concentrations of estrogens and progesterone within the ovary at the time of ovulation may influence the responsiveness of ovarian smooth muscle cells to prostaglandins and adrenergic mediators, substances naturally occurring in the ovary.

Oviductal muscular activity and sensitivity to neurohypophyseal hormones decline during the late proliferative phase. Estrogen treatment can duplicate this effect. The simultaneous administration of progesterone and estrogen produces no perceptible difference from the effects found with estrogen alone.[15] Estradiol has been shown to decrease the sensitivity of rat uterus in vivo to prostaglandins, while progesterone alone has no effect; both steroids, however, decrease the sensitivity of the rat uterus to several drugs in vitro.[19]

As indicated in the present study, a prominent ovarian contractile pattern was found after progesterone administration. Furthermore, the response to adrenergic agents, and especially to prostaglandins, was appreciably enhanced when compared with contractility after estrogen treatment. We cannot exclude the possibility that the action of estrogens and progesterone on ovarian contractility may depend upon whether the experiment is carried out in vivo or in vitro.

It has postulated that hormones affect cell function by altering the concentration of cyclic AMP within individual cells.[20] With reference to smooth muscle activity, ample evidence indicates that hormones which inhibit contractility provoke an increased concentration of cyclic AMP within the cell.[21] Within the framework of this study, we are unable to establish whether the enhanced ovarian contractility observed after progesterone treatment can be related to a direct effect of the hormone or to a relative lack of estrogen during the administration of progesterone. Conjugated estrogens were given in an aqueous solution; the three-week treatment with this preparation may have been sufficient to permit metabolism of administered estrogens. On the other hand, the dose of progesterone was sufficiently high to inhibit the release of gonadotropins and probably prevent further estrogen biosynthesis by the remaining ovary. To interpret our results in terms of the dynamics of cyclic AMP, estrogens appear to inhibit ovarian contractility; during progesterone administration, estrogens may be displaced from receptor sites in ovarian smooth muscle cells, allowing intracellular cyclic AMP concentration to return to previous levels. This alteration may result in the more pronounced contractility observed during the second phase of the experiment, following progesterone treatment. An alternate consideration is that progesterone itself produces decreased cyclic AMP within the cell and enhances ovarian contractility. As indicated above, it has been suggested that hormones which result in decreased concentrations of cyclic AMP in smooth muscle may result in increased contractile activity

of the cell.[22] Although no previous data are available concerning the action of cholinergic drugs upon ovarian contractility, the fact that the responses to cholinergic agonists were inhibitory following estrogen treatment and stimulatory after progesterone may also be related to changes in concentration of cyclic AMP, and emphasizes the importance of the specific hormonal milieu.

Ovarian contractile action has been postulated as a significant factor in the mechanism of ovulation. Prostaglandin $F_2\alpha$ has been shown to increase ovarian contractility.[5,6] Indomethacin, an inhibitor of prostaglandin biosynthesis, prevents follicular rupture, but not follicular maturation, in rats and rabbits.[23-26] During a normal menstrual cycle in primates, estrogen concentration drops at midcycle, and, as Kirton et al[27] have demonstrated, progesterone begins to rise at about the time LH peaks. These hormonal fluctuations may provoke changes in cyclic AMP within smooth muscle cells in the ovarian stroma, increasing their responsiveness to endogenous adrenergic mediators or prostaglandins locally present at that particular time. Studies are currently underway in our laboratory to determine whether or not an increase in ovarian $PGF_2\alpha$ and/or norepinephrine concentration occurs at the time of ovulation in the rabbit.

No single hypothesis at present can explain the precise mechanism of ovulation, but data have accumulated to suggest the involvement of specific local factors. Ovarian contractions have been clearly demonstrated; they seem to be influenced by estrogens and progesterone. Several humoral agents naturally present in the ovary can modify ovarian contractility. It is likely that concentrations of hormones, prostaglandins, and/or autonomic agents reach a critical balance within the ovary at the approximate time of ovulation. This appropriate environment may foster optimal contractility of the ovary and resultant rupture of the mature follicle with ovum expulsion.

SUMMARY

Five female rhesus monkeys were treated with natural estrogens, 5 mg/day for three weeks, after which ovarian contractility was studied in vitro in one of the ovaries. Estrogen treatment was followed by progesterone, 25 mg/day for three weeks, after which the contractility of the remaining ovary was similarly measured. Responses to autonomic agents and prostaglandins were studied in both groups.

Spontaneous ovarian contractility and ovarian contractile responsiveness to prostaglandins and norepinephrine were found to be enhanced after progesterone treatment. Cholinergic agonists had a stimulatory effect after progesterone and an inhibitory effect after estrogens. Our results suggest that ovarian contractile responsiveness is modified by the local steroid environment, perhaps through intracellular changes in cyclic AMP.

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