Prostaglandin E\textsubscript{2} Induces Receptive Behaviors in Female
\textit{Xenopus laevis}

\textbf{Andrea S. Weintraub,* Darcy B. Kelley,*
and Richard S. Bockman†}

*Department of Biological Sciences, Columbia University, New York, New York 10027,
and †Department of Medicine, Cornell University Medical College and Memorial Sloan-Kettering Cancer Center, New York, New York 10021

The object of this study was to examine the effects of exogenous and endogenous prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) on the sexual behavior of female South African clawed frogs, \textit{Xenopus laevis}. Ticking and leg extension, which communicate sexual unreceptivity to males, were studied in intact, ovariectomized, and ovariectomized-oviductectomized females. Injection of nanomolar amounts of PGE\textsubscript{2} into the dorsal lymph sac significantly increased receptivity in intact, ovariectomized, and ovariectomized-oviductectomized females. The onset of the PGE\textsubscript{2} behavioral effect occurs within 30 sec to 3 min of injection for intact and ovariectomized females; for ovariectomized-oviductectomized females, the latency period for the effect ranges from 10–20 min. PGE\textsubscript{2} induced receptivity in doses as low as 0.03 μg/frog. Injection of the prostaglandin synthesis inhibitors, indomethacin and flurbiprofen (FBP), blocked chorionic gonadotropin-(HCG-) induced behavioral receptivity, suggesting that endogenous prostaglandin synthesis may have a role in regulating female sexual behavior. Flurbiprofen blockade of HCG-induced receptivity was reversed by PGE\textsubscript{2} administration, suggesting that FBP's effects are PG synthesis-specific. © 1985 Academic Press, Inc.

During the breeding season, South African clawed frogs, \textit{Xenopus laevis}, exhibit bouts of spawning triggered by rainfalls and resultant drops in temperature (Kalk, 1960). Female reproductive behaviors can also be observed under laboratory conditions (Russell, 1954; Kelley, 1982) when exogenous hormone treatments are administered. Receptivity can be induced in reproductively active, intact females 6 to 10 hr after administration of exogenous gonadotropins; longer treatment (several days to a week) is required when the ovaries are regressed (Deuchar, 1975). Ovariectomy abolishes sexual receptivity which can be reinstated by several weeks of combined estradiol/progesterone treatment (Kelley, 1982).

Neither gonadotropin nor ovarian steroids appear to act with sufficient rapidity to function as potential endocrine mediators of the rainfall-induced spawning that occurs in the wild. One candidate for this role is pros-
taglandins (PG's), hydroxylated fatty acid metabolites of membrane-derived arachidonate, which are present in diverse tissue types, including female reproductive organs (Samuelsson, 1969; Horton, 1969). Our experiments on *X. laevis* were inspired by the work of Stacey and colleagues (Stacey, 1976; Stacey and Peter, 1979) who have shown that exogenous and endogenous prostaglandins rapidly induced receptivity in female fish as they do in many other female vertebrates (Buntin and Lisk, 1979; Chob-sieng, Naor, Koch, and Lindner, 1975; Diakow and Nemiroff, 1981). Prostaglandins have also been shown to inhibit receptivity in some species; the direction of PG effects may be related to changes in female sexual receptivity induced by mating (Tokarz and Crews, 1981; Marrone, Rodriguez-Sierra, and Feder, 1979).

We report here that prostaglandin E₂ (PGE₂) rapidly (within seconds) and reliably produces receptive behaviors in female *X. laevis* at doses as low as 0.03 µg. Neither the ovaries nor the oviducts are necessary to obtain the response to the hormone, suggesting that the target of PGE₂ is extragonadal. A single injection of a prostaglandin synthesis inhibitor can delay the onset of gonadotropin-induced receptivity by 6 hr, indicating a possible endogenous role of PGs in the regulation of female reproductive behaviors.

**GENERAL METHODS**

*Female behaviors.* Sexually unreceptive female *X. laevis* respond to a clasp assault by a sexually active male with extension of the hind legs and a ticking vocalization functionally similar to the “release call” of other anurans (Russell, 1954; Kelley, 1982). Under conditions of constant light and temperature, laboratory-housed females are sexually unreceptive throughout the year, although we have noted a slight increase in receptivity during the summer months. Sexually receptive females respond to a male clasp attempt with exaggerated knee flexion and thigh abduction, and do not vocalize.

*Subjects and maintenance.* Adult, reproductively mature male and female *X. laevis* were selected from our colony for use in these experiments. Males were randomly chosen, whereas females were selected from a pretested population of females known to “tick” reliably in response to clasp assaults by mature males. Males were obtained from the Nasco Company (Fort Atkinson, Wis.), females from either Nasco or Kelly Evans (Ann Arbor, Mich.). Frogs were maintained on a 15 hr light/9 hr dark cycle (light 10:00 P.M. to 1:00 P.M.) at approximately 22° C, and were housed in 47 × 23 × 14-cm tanks containing a solution of 0.4% saline in standing tap water, with 2–4 same sex cohorts per home tank. All subjects were fed a standard frog chow (“frog brittle,” Nasco) three times a week and after feeding, the tank water was changed. No disease was apparent in any of these subjects during the course of the study.
Surgical procedures. Females were ovariectomized after anesthetization in MS-222 (tricane methane sulfonate, 0.23 mg/200 ml tap water; Aldrich Co.). A single midline abdominal incision was made, the underlying muscle and fascia were incised, and the ovaries and associated fat bodies were exposed. The blood vessels to the ovaries were ligated, and the ovaries, egg mass, and associated fat bodies were excised. The muscle, fascia, and skin were then sutured. Surgically treated animals were given a 2-week recovery period before testing was resumed. Females for oviduct removal were ovariectomized at the same time to prevent the typically fatal consequences of severing the blood vessels that run in the mesentery between the two organs. There were only two deaths associated with postoperative complications, one from ovariectomy and one from combined ovariectomy–oviductectomy. Control females were not sham operated because previous studies (Kelley, 1982) had shown that superficial surgery (operations, ovary extrusion and replacement) does not affect sexual receptivity; removal of equivalent-sized nonreproductive organs (i.e., liver or kidney) would have grossly debilitated the animals.

Hormones and drugs. Lyophilized chorionic gonadotropin (HCG; Sigma Chemical Co.) was dissolved in sterile distilled water to make an injection solution of 100 international units in 0.5 ml. Prostaglandin E₂ (PGE₂; Sigma Chemical Co.) was dissolved in absolute ethanol to make a 1-mg/ml stock solution; the desired dose of PGE₂ for testing was taken from this stock and delivered in 0.5 ml of injection vehicle (0.45 ml distilled water, 0.05 ml absolute ethanol). Flurbiprofen (Boots Ltd.) or indomethacin (Sigma Chemical Co.) was dissolved in ethanol and diluted in sodium bicarbonate-buffered sterile water to make effective dosages of $10^{-6} M$ and $10^{-5} M$, respectively. All hormones and drugs were administered by injection into the dorsal lymph sac of the frogs. Control injections consisted of 0.5 ml of the appropriate injection vehicle. No mortality was associated with any hormone or drug administration.

General testing procedure. Four or six male/female pairs of frogs were observed simultaneously for 90-min sessions on 2 consecutive days (one test sequence). In the late afternoon prior to Day 1 of testing, each male received HCG in order to increase the amount of time spent calling (Wetzel and Kelley, 1983) and the frequency of clasp attempts (Kelley and Pfaff, 1976). The male was then placed in a 51 × 27 × 32-cm aquarium lined with 5 cm of thick foam padding and a polyethylene bag and filled with approximately 20 liters of standing tap water. Each of these aquaria was equipped with a Wilcoxon hydrophone (Model H505) suspended from a plexiglass cover to a height of approximately 10 cm from the bottom of the tank; these hydrophones were connected to Akai tape recorders (Model GX-255). Males received HCG again on the morning of each day. Females received their injections at the onset of the dark cycle (1:00 P.M.). After injection, females were placed in the recording
tanks with the males and allowed to remain undisturbed in the dark for 30 min before testing was begun. All observations on leg extension were conducted under red light illumination so as to simulate the natural environmental conditions as closely as possible and to minimally disrupt the subjects' normal interactions. All vocalizations were recorded during the test sessions on reel-to-reel magnetic audio tapes (Scotch 207).

Measuring female receptivity. To assess receptivity from postural responses, the occurrence and duration of each clasp of a female by a male was noted. Note that a single clasp assault by a male of a sexually receptive female typically results in a prolonged amplexus of up to 72 hr (Kelley, 1982). Female response to clasping was scored as previously described (Kelley, 1982). The maximally flexed leg position of receptive females was assigned a score of 0. In contrast, unreceptive females respond by fully extending the hind limbs; such an extended position was scored as 2. Any leg posture intermediate between maximum thigh abduction and maximum leg extension received a score of 1. For each test period, the leg extension scores observed were averaged across clasp assaults. These means were used to characterize the receptive state of individual females during a given test. Changes in receptivity were compared across treatments for individual frogs; each female served as its own control. The direction of change in leg extension scores of individual females was evaluated using the appropriate nonparametric statistics (Siegel, 1956). Since female leg extension is inversely proportional to receptivity, we derived a receptivity score from the leg extension data. The receptivity score is calculated by multiplying the leg extension score (LES) by 50 and subtracting this product from 100; thus, a LES of 0 corresponds to 100% receptivity, while a LES of 2 is equivalent to 0% receptivity.

Receptivity was also measured by examining the tickling response of females. Audio recordings of the frog vocalizations during the 90-min sessions were analyzed to determine whether or not the females ticked in response to male clasp attempts for each of the different treatment conditions. The amount of time the females spent tickling and the percentage of females ticking were compared across treatment conditions.

The present experiments were designed to measure female receptivity, defined as a behavioral response (leg flexion, silence) to a clasp assault by a male. If our experimental manipulations had also affected female attractiveness, we would expect to see changes in the frequency of clasp attempts by males (Kelley, 1982). Any test sequence without a single clasp attempt yields no data on female receptivity. We therefore maximized male clasping by HCG injection (Kelley and Pfaff, 1976). Gonadotropin-injected males are insensitive to the attractiveness of females. Whereas intact, uninjected males rarely attempt to clasp an ovariectomized (unreceptive) female and release such a female rapidly, HCG-injected males
exhibit repeated and persistent clasp assaults (Kelley and Pfaff, 1976). Thus one result of the use of HCG-injected males in the present study is that any subtle PGE$_2$-induced changes in attractivity would not be detected. Dramatic changes that might be attributed to unattractive females (e.g., no clasp attempts in a test sequence) were extremely rare in the present study and not consistently associated with any endocrine manipulation.

**SPECIFIC METHODS AND RESULTS**

Pilot experiments comparing the common prostaglandins (PGE$_2$ and PGF$_{2\alpha}$) indicated that PGE$_2$ was more effective in promoting female receptivity. All experiments therefore utilized PGE$_2$.

**Intact Females**

*Methods.* The behavioral effect of PGE$_2$ on sexual receptivity in 12 intact female *X. laevis* was compared to the effect produced by a control solution in the same subjects. In Test Sequence 1, females received vehicle injection on Day 1, and a 12-µg dose of PGE$_2$ on Day 2. In Sequence 2, females received vehicle on both days as an internal control for any behavioral changes resulting from 2 consecutive days of testing and for any endogenous fluctuations in receptivity.

*Results: Leg extension.* Injection of PGE$_2$ resulted in a rapid onset of receptivity, between 30 sec and 3 min of injection. In Fig. 1 (left-hand panel), the mean receptivity scores of intact females given PGE$_2$ are compared with the receptivity scores of these same females after injection of vehicle. Injection of PGE$_2$ on Day 2 significantly increased the receptivity scores from 4% on Day 1 to 98.5% (Sign test, $P < 0.001$). In comparison, there was no significant change in the receptivity scores when females received vehicle on both days.

![Fig. 1](image)

*Fig. 1.* The effect of injection of vehicle (V) and PGE$_2$ on the receptivity scores of intact and ovariectomized females is shown as a function of injection sequence. Injection of 12 µg of PGE$_2$ significantly increased female receptivity ($p < 0.001$, Sign test); injection of vehicle did not.
Fig. 2. The effect of injection of vehicle (V) and PGE$_2$ on ticking in intact and ovariectomized females is shown as a function of injection sequence. Injection of 12 µg of PGE$_2$ induces complete suppression of ticking in all subjects tested that had ticked with vehicle on the previous day.

_Ticking._ All subjects ticked when injected with vehicle; no females ticked when injected with PGE$_2$ (Fig. 2; left-hand panel). When females received vehicle on both test days, there was a small and statistically nonsignificant decline in the percentage of females that ticked on Day 2 ($P > 0.05$, Sign test). When data on total time spent ticking were compared for the two days of vehicle injection, the Day 1 value was 38.6 min and the Day 2 mean value was 5.3 min. This decrease in time spent ticking was statistically significant ($P < 0.001$, Sign test). Thus, it appears that 2 consecutive days of testing with a mature male somewhat decreases the number of females ticking and shortens the amount of time that each individual female ticks.

_Ovariectomized Females_

In this and the following experiment, we examined the hypothesis that either the ovaries or the oviducts are the target mediating the behavioral effects of exogenously administered PGE$_2$.

_Me_\text{th}o_d_s._ Eleven females from the initial study were ovariectomized and retested 2 weeks postoperatively, following the standard test-sequence protocol. In Sequence 3, females received vehicle on both days, as an internal control, while in Sequence 4, subjects ($N = 10$) received vehicle on Day 1 of testing and 12 µg of PGE$_2$ on Day 2.

\textit{Results: Leg extension.} As with the intact subjects, injection of PGE$_2$ powerfully enhanced receptivity. Ovariectomized females had receptivity scores of 0% following vehicle injection; receptivity increased to 100% after PGE$_2$ administration (Fig. 1, right-hand panel; differences across treatments for individuals, $P < 0.001$, Sign test). In comparison, there was virtually no change in receptivity when females received vehicle on both days (1% on Day 1 vs 2.5% on Day 2).

_Ticking._ Complete suppression of ticking after PGE$_2$ injection was observed in ovariectomized frogs. Again a small and nonsignificant decline
in the percentage of vehicle-injected females that ticked on Day 2 compared to Day 1 was noted (Fig. 2, right-hand panel; 72.7% on Day 1 vs 54.5% on Day 2). The amount of time vehicle-injected females spent ticking on Day 2 as compared to Day 1 also decreased nonsignificantly (mean Day 1, 10.1 min; mean Day 2, 3.8 min).

Ovariectomized–Oviductectomized Females

From the previous experiment, it appeared that the ovaries are not the target of PG action. We then turned to the oviducts.

Methods. Eleven experimentally naive, sexually mature females were tested following the standard test-sequence protocol. The subjects were then ovariectomized–oviductectomized and retested 2 weeks postoperatively.

Results: Leg extension. Injection of PGE₂ induced receptivity in both intact and ovariectomized–oviductectomized subjects. When intact, the mean female receptivity score was 1% following vehicle injection on Day 1. Receptivity rose to 100% following PGE₂ injection on Day 2. When ovariectomized and oviductectomized, the same females were 20% receptive on Day 1 following injection of vehicle; administration of PGE₂ on Day 2 elevated receptivity to 100%. The time required for PGE₂ to induce a receptive leg position increased from the 0.5- to 3-min interval of the intact condition to 10–15 min in the ovariectomized–oviductectomized condition. The duration of the response was similar to that of the intact females (8–12 hr).

Tickling. Following PGE₂ administration, no females ticked in either intact or ovariectomized–oviductectomized conditions. The percentage of females ticking after vehicle injection on Day 1 declined following surgery. The frogs also displayed unusually vigorous escape movements in response to handling. The latency between PGE₂ injection and suppression of ticking increased to 10–20 min as compared to the 5- to 10-min interval when the females were intact.

Dose–Response Effectiveness of PGE₂

Since PGE₂ proved to be a powerful inducer of receptivity at a 12-μg dose, we wished to investigate the potency of this effect at lower doses, using some of the same subjects as in the previous experiments. Preliminary radioimmunoassays of PGE₂ levels in female blood suggested circulating levels in the range of 5 ng/ml. We therefore injected doses down to approximately the physiological level (assuming 5 ml of blood total volume, a 0.03-μg dose should result in 6 ng/ml blood). However, any inactivation of PGE₂ would result in a lower effective dose as would delay in delivery from injection site to the circulatory system.

Methods. Twelve females (10 ovariectomized, 2 intact) were tested in the standard 2-day sequence. No difference in the dose effectiveness of PGE₂ on receptivity of ovariectomized vs intact frogs was noted. At the
start of these studies, females had been ovariectomized for approximately 4 months. Of the four females tested simultaneously in each test sequence, two were arbitrarily selected to receive PGE₂ first, while the other two received vehicle first. No difference in results due to the order of drug administration was noted. Weights of the subjects ranged from 40–60 g (mean = 50 g). The dose of PGE₂ administered in each successive sequence was halved and then decreased by an order of 10 until the point was reached where vehicle and PGE₂ produced virtually identical receptivity scores. That dose was then multiplied by 5 for the next test sequence. At the conclusion of dose–response studies, females had been ovariectomized for approximately 8 months. In the initial work presented above, the duration of the PGE₂ effect is brief; the onset occurs between 30 sec and 3 min of injection, and the effect was not seen to persist longer than 12 hr. Since the test sequences in this study were separated by at least a week’s time, we do not feel results could be confounded by any effect of lingering, circulating PGE₂. Repeated administration of PGE₂ might, however, have cumulative effects on PGE₂ targets which could alter receptivity. These experiments were designed to detect any such effects since scores of females when vehicle-injected could be compared across test sequences. No significant differences in receptivity scores of vehicle-injected females were obtained. We conclude that results of these dose–response studies were probably not confounded by repeated testing.

Results: Leg extension. Figure 3 illustrates the effectiveness of different PGE₂ doses on mean receptivity scores of 12 female X. laevis as compared to the effect of vehicle on receptivity for these same subjects. As the dose of PGE₂ was decreased from 12 to 6 to 0.6 and finally to 0.06 μg, female receptivity remained constant between 95 and 100%. The corresponding receptivity for vehicle during these test sequences ranged from 2–3%. It was not until the dose was reduced to 0.006 μg/per animal that there was no significant difference between the PGE₂-induced receptivity score and the vehicle-induced receptivity score (9% for both

![Bar chart showing the effectiveness of various PGE₂ doses on receptivity scores of intact and ovariectomized females. PGE₂ is effective in inducing receptivity in the dose range of 12–0.03 μg. At a 0.006-μg dose of PGE₂, there is no significant difference compared to vehicle.](image-url)
treatment conditions). At all higher doses, there were significant differences between vehicle and PGE$_2$ treatment conditions (Sign test, $P < 0.001$). When the dose was raised to 0.03 from 0.006 $\mu$g, females once again became 100% receptive. The receptivity score associated with injection of vehicle during this sequence remained at 10%.

**Tickling.** Low doses of PGE$_2$ were less effective in complete suppression of tickling than in inducing full leg flexion. Figure 4 compares the effects of vehicle and various doses of PGE$_2$ on the percentage of females ticking. At the 12-$\mu$g dose, the 100% suppression of ticking previously demonstrated was replicated. However, in the dose range from 6.0–0.06 $\mu$g, 20% of the females ticked. When the dose was decreased further to 0.006 $\mu$g, 40% of the females ticked; this percentage was not significantly different from that displaying ticking with vehicle (range = 40 to 80%). When the dose was increased to 0.03 $\mu$g, the number of females that ticked returned to the value obtained for the 6.0- to 0.06-$\mu$g dose range.

**Prostaglandin Synthesis Inhibitors and HCG-Induced Receptivity**

The studies described above suggested that *exogenous* prostaglandins act rapidly to promote receptivity in female *X. laevis*. In addition we wished to determine whether *endogenous* prostaglandins are involved in modulation of female sexual behavior. In particular, we wished to test the hypothesis that our administration of PGE$_2$ mimicked ovulation-induced secretion of that hormone, an effect described previously in mammals and fish (Behrman, 1979; Stacey and Goetz, 1982). Ovulation, and subsequent sexual receptivity, were induced by administration of human chorionic gonadotropin. Two inhibitors of prostaglandin synthesis were used: flurbiprofen (FBP) and indomethacin (IM). The final effective (assuming distribution in 100 ml of bodily fluids) doses chosen were $10^{-5}$M for IM and $10^{-6}$M for FBP. Neither drug resulted in any apparent morbidity; feeding was not disturbed.

![Graph](image)

**Fig. 4.** The dose–response of PGE$_2$ on tickling in intact and ovariectomized females. PGE$_2$ suppressed tickling over the dose range of 12–0.03 $\mu$g. At a 0.006-$\mu$g dose, PGE$_2$ is no more effective in suppressing tickling than vehicle.
Our choice of behavioral observation times was determined by the temporal effectiveness of HCG administration. Behavioral receptivity is maximal 4 to 6 hr after HCG injection (Kelley, 1982). In vitro studies of FBP effects on prostaglandin synthesis reveal inhibition to be maximal 5 to 7 hr following administration. Our behavioral observation period falls within this time frame.

**Indomethacin: Methods.** The effects of IM pretreatment on HCG-induced sexual receptivity were compared to effects of vehicle pretreatment. Each injection of indomethacin consisted of 0.5 ml of a 10⁻⁴ M sodium-bicarbonate-buffered solution. Frogs were tested for 4 successive days, two test sequences, first with vehicle pretreatment and then with IM pretreatment. The vehicle or IM pretreatment preceded the HCG injection by 1 hr; behavioral observations (receptive leg position only) were made 4 hr later. In order to assess possible effects of IM on sexual receptivity of intact non-HCG-treated females, 6 of the 12 frogs were retested the next week with vehicle followed by IM injection (IM-V test sequence).

**Results.** The mean receptivity score for vehicle-HCG-treated females was 92.5%. For these same females when IM-HCG-treated, the mean receptivity score was 51.5%. When scores of individual females were compared across treatment conditions, receptivity scores following IM-HCG were significantly (P < 0.005) lower than following HCG alone. In the IM-V test sequence, the mean receptivity score was 25%. Receptivity scores of IM-V-treated females were always less than (N = 4) or equal to (N = 2) scores obtained following V-HCG or IM-HCG treatment.

One possible confounding variable in the V-HCG, IM-HCG test-sequence results is the effect of repeated testing. Previous studies, however, have shown that receptivity, if anything, increases with successive test days (Kelley, 1982). We therefore conclude that indomethacin pretreatment blocks HCG-induced receptivity. Receptivity was restored approximately 6 to 8 hr after the last IM injection. IM injection also decreased receptivity scores in untreated (i.e., non-HCG-injected) females.

**Flurbiprofen: Methods.** Eighteen intact, sexually mature, experimentally naive female X. laevis were observed with sexually active males for three 2-day test sequences. The standard experimental protocol was followed. For the first test sequence, females received an injection of vehicle followed 1 hr later by an injection of HCG. The following week, an injection of flurbiprofen (FBP; 10⁻⁵ M in 0.5 ml buffered 0.65% saline) was substituted for the vehicle injection. For the third test sequence (which followed Test Sequence 2 immediately), females were injected with vehicle followed by HCG.

We wished to determine whether PGE₂ injection restores the sexual receptivity of FBP-treated females. Twelve of the above frogs were first tested with PGE₂ alone (60 μg), then with FBP followed by PGE₂, and finally with FBP followed by a combination of PGE₂ and HCG.
**Results.** Injection of the prostaglandin synthesis inhibitor, flurbiprofen, reduced the sexual receptivity of HCG-injected females. The mean receptivity scores were less following the FBP–HCG injection (39.1%) than in the preceding vehicle–HCG test sequence (81.8%; Sign test on individual comparisons, \( P < 0.02 \)). There was also a tendency for the FBP–HCG receptivity scores to be less than scores during the succeeding vehicle–HCG test sequence (64.4%), but this difference was not significant (\( P > 0.05 \)). In all FBP-injected females then injected with PGE$_2$, the receptivity scores were 100%.

The oviposition of six females was followed in order to determine whether FBP prevents egg release. All females oviposited in response to HCG injection. Eggs were also observed in the tanks following the FBP–HCG injection schedule.

**DISCUSSION**

Taken together, the data suggest that prostaglandins are involved in the sexual receptivity of female *X. laevis*. We have demonstrated that moderate-to-low doses of exogenous PG are powerful and swift promoters of receptivity in these females, as indicated by the shift from unreceptive to receptive leg positions and by the suppression of ticking. The extent of sexual receptivity induced by exogenous PG is not dependent on the presence of the ovaries and/or oviducts. PGs may mediate gonadotropin-induced receptivity, since injection of the PG synthesis inhibitors, flurbiprofen and indomethacin, block HCG-induced behavioral receptivity.

**PG Effects on Female Sexual Behavior in Other Species**

PGs have been implicated in the regulation of reproductive behavior in many amphibian, teleost, and mammalian species. Diakow and Nemiroff (1981) have suggested that PGs are involved in the inhibition of the release call in the anuran *Rana pipiens*. Diakow (1978) confirmed Noble and Aronson’s hypothesis that it is the rise in intraabdominal pressure, caused by the presence of ovulated eggs, which inhibits the release call. Whether or not there is a concomitant fluid accumulation, this distension and elevation of intraabdominal pressure effectively suppresses release calling (Diakow, 1978). Diakow and Nemiroff (1981) showed that arginine-8 vasotocin (AVT) may inhibit release calling by increasing fluid retention and thus intraabdominal pressure. Since PGs were also found to suppress release calling in ovariectomized females, and since the AVT effect on female receptivity can be blocked by indomethacin, a PG synthesis inhibitor, it has been suggested that AVT’s action in inducing female receptivity may be mediated by endogenous release of PGs. Ticking in *X. laevis* probably functions as a release call (Kelley, 1982) and thus is a behavior comparable to the *Ranid* vocalization.

Stacey (1976) proposed that PGs that induce spawning behavior in
goldfish and other teleosts function as fast-acting, short-term regulators of female sexual behaviors in these species. He has postulated that PGs are released from the ovaries and/or oviducts in response to the presence of ovulated eggs in the female reproductive tract. PGs also facilitate receptive behaviors in higher vertebrates (e.g., lordosis in ovariectomized, estrogen-primed rats) (Rodriguez-Sierra and Komisaruk, 1977; Marrone, Rodriguez-Sierra, and Feder, 1979; Buntin and Lisk, 1979).

**Mechanism of PG-Induced Receptivity**

Several important questions remain. What is the target of PG action? Is our exogenous administration of PG evoking a physiological response that mimics an endogenous process? If so, what is the endogenous source of PG?

**Target**

The site of PG action is not known. We report that PG-induced behavioral changes can begin within 30 sec of PG injections in intact and ovariectomized females; similar findings have been reported by Rodriguez-Sierra and Komisaruk (1977) in rats and by Stacey and Peter (1979) in teleosts. The rapidity of this response suggests a neurally mediated mechanism. PGs may act locally on accessory structures in the female reproductive tract and stimulate afferent activity to the CNS or may act directly on CNS targets involved in reproductive behavior. To this end, our experiments have ruled out the ovaries and oviducts as the sole PG target tissue, as the behavioral responses of short- (2 weeks) and long- (4 to 8 months) term ovariectomized and ovariectomized–oviductectomized females were identical to those of intact frogs. However, we note that the latency of PG-induced receptivity was longer in oviductectomized females, hinting at a role for the oviduct in PG-induced receptivity. This temporal difference in the onset of receptivity must be viewed with caution; these females were more irritable than animals that had received ovariectomy alone. Perhaps if tested again several months postoperatively, the time required for PG to induce receptivity in oviductectomized frogs would fall to levels observed for the other groups of animals tested.

There are several lines of evidence suggesting that PGs can act directly on the CNS to affect neuroendocrine secretion and behavior necessary for reproduction (e.g., Behrman, 1979; Ojeda and McCann, 1978). In fish, PGs are effective at much lower doses if placed directly into the CSF or brain tissues (Stacey and Peter, 1979). In isolated frog spinal cord, PGs have been shown to depolarize motor neurons (Phillis and Tebecis, 1968). In *X. laevis*, PGs might be acting directly on the flexor motor neurons innervating the thigh muscles that are responsible for the leg position (thighs abducted, knees flexed) that conveys receptivity.
Source

We report here that IM and FBP (inhibitors of prostaglandin synthesis) block HCG-induced behavioral receptivity. This finding suggests that endogenous PGs may be involved in gonadotropin-stimulated sexual receptivity of female *X. laevis*. The source of endogenous PGs may be the CNS itself. Exogenous injection of PG would then mimic PG release within or between neurons. Alternatively, endogenous PGs may be produced in peripheral reproductive tissue. Immunocytochemical studies (unpublished) reveal the presence of abundant PG synthesis in ciliated glandular epithelial cells in the proximal and distal portions of the oviductal lumen of female *X. laevis*.

Conclusions

In the wild, female *X. laevis* engage in multiple bouts of spawning throughout the breeding season; a portion of the egg mass is released with each amplexus (Kalk, 1960). Once ovulated, the eggs travel through the oviducts and are extruded. Unlike other frogs, *X. laevis* do not store ovulated eggs in a precloacal "uterus." Once the eggs undergo the final reduction division of meiosis and begin traversing the oviduct, it is essential that the female be sexually receptive. Our hypothesis is that PG release, stimulated by egg–oviduct interactions, ensures sexual receptivity by acting on CNS targets for reproductive behaviors. Such a mechanism is not necessarily confined to *Pipids* but may operate in other anurans (see Diakow, Scharff, and Aronow, 1983) and perhaps even in mammals.

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