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Clonidine Inhibits Female Spawning Behavior in Ovulated and Prostaglandin-Treated Goldfish

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STACEY, N E *Clonidine inhibits female spawning behavior in ovulated and prostaglandin-treated goldfish* PHARMACOL BIOCHEM BEHAV 20(6) 887–891, 1984 — Previous studies have indicated that female spawning behavior in goldfish is stimulated by prostaglandin (PG) synthesized in the reproductive tract shortly after ovulation and acting within the brain In this study, clonidine inhibited PG-induced spawning behavior of nonovulated female goldfish in a dose-dependent manner and also inhibited spawning of ovulated goldfish. In male goldfish, clonidine also inhibited PG-induced female spawning behavior but did not affect male spawning behavior The results provide further support for a role of endogenous PG in female spawning behavior of goldfish and are consistent with the findings [15] that clonidine antagonizes the action of PG on sexual behavior in the female guinea pig

Clonidine Prostaglandin Spawning behavior Goldfish

PROSTAGLANDINS (PGs) have been shown to exert rapid effects on female reproductive behaviors in a variety of vertebrates including mammals, reptiles, amphibians and teleosts. Stimulatory effects on female sexual behavior were first reported in the rat [13], and have since been observed in the hamster [2], the leopard frog, *Rana pipiens* [9], *Xenopus laevis* [16], the goldfish, *Carassius auratus* [20], the paradise fish, *Macropodus opercularis* [27], and a cichlid, *Aequidens portalegrensis* [6]. Inhibitory effects of PG on female sex behavior have been demonstrated in both the guinea pig [18] and the green anole lizard, *Anolis carolinensis* [26]. Although there is evidence that PGs act within the brain to affect female sexual responsiveness in the rat [12], *Anolis* [26], and the goldfish [25], the mechanism of action of PGs on female sexual behaviors has been investigated only in the guinea pig. Blood levels of prostaglandin F_2 alpha (PGF) and its major metabolite, PGFM, increase soon after copulation in the guinea pig [15]; female sexual receptivity is rapidly inhibited both by coitus [11] and by exogenous PG [18]. The findings that abbreviation of receptivity following either copulation [14] or PG treatment [15] is blocked by the alpha-adrenergic agonist, clonidine, have led Irving *et al* [15] to suggest that PG released from the genital tract in response to the mechanical stimulation of coitus inhibits receptivity by interfering with an alpha-adrenergic mechanism. The present study was carried out to determine whether clonidine also antagonizes the stimulatory action of PG on female sexual behavior in the goldfish.

METHOD

Animals and Maintenance

Common goldfish were purchased in April–June, 1983, from Grassyforks Fisheries Co., Martinsville, IN, and kept for several months on a 16L:8D photoperiod in flow-through stock tanks at 20–22°C (Experiments 1 and 2) or 12–14°C Experiment 3). Several days prior to behavioral testing, fish were removed from stock tanks, anesthetized in 2-phenoxy-ethanol (0.05%, Syndel), weighed, fin-clipped for identification, and placed in 80 l flow-through holding aquaria (20–22°C, 16L:8D; 3 fish per aquarium) provided with a gravel substrate. In Experiment 3, the holding aquaria were at 14°C at the time of fish transfer, and were subsequently warmed to 20–22°C over a period of several hours. Fish in stock tanks were fed ad lib with Ewos pellets and Nutrafin flaked food (R. C. Hagen, Montreal); fish in holding aquaria received only Nutrafin. Females in Experiment 1 were in a post-spawning condition with regressed ovaries, and males in Experiment 2 were in a similar condition with barely visible tubercles (pearl organs) on the opercula and pectoral fins. Females in Experiment 3 were in a preovulatory condition, and ovulated spontaneously on the morning of the behavioral test in response to elevated water temperature and the addition of artificial aquatic vegetation (bundles of green acrylic yarn) at the time of transfer [22]

Drugs

Clonidine hydrochloride (Catapres, Boehringer Ingelheim) was dissolved in 0.6% NaCl at several concentrations (0.04–2.0 mg/ml) immediately before administration, and injected intraperitoneally (IP) at dosages from 0.1 to 5.0 μg/g (2.5 μl/g) body weight. Prostaglandin F2 alpha (PGF, Tris salt, Sigma), previously frozen in small aliquots in ethanol (20 mg/ml), was diluted to 1 mg/ml with phosphate buffer immediately prior to intramuscular (IM) injection with a Hamilton microsyringe at a dose of 50 ng/g (0.05 μl/g) body weight. Fish were not anesthetized for injection

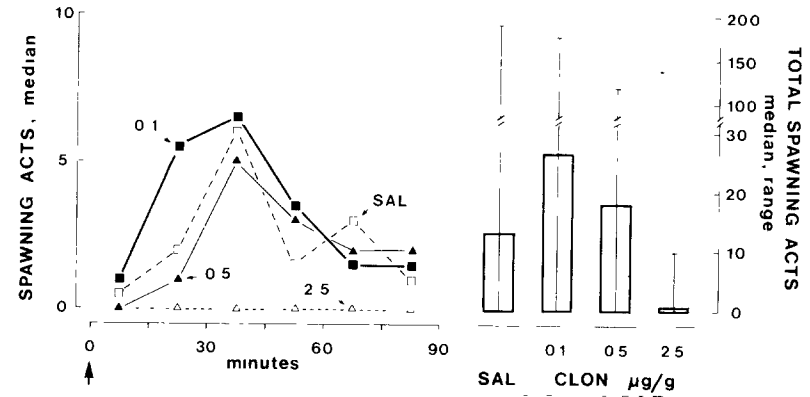

FIG 1 Effect of clonidine (0 1, 0 5, and 2 5 μg/g IP, arrow) on female spawning behavior of nonovulated goldfish injected with prostaglandin F2 alpha (50 ng/g IM, arrow) The highest clonidine dose (2 5 μg/g) significantly inhibited spawning behavior ($p<0$ 02) N=12 in all groups

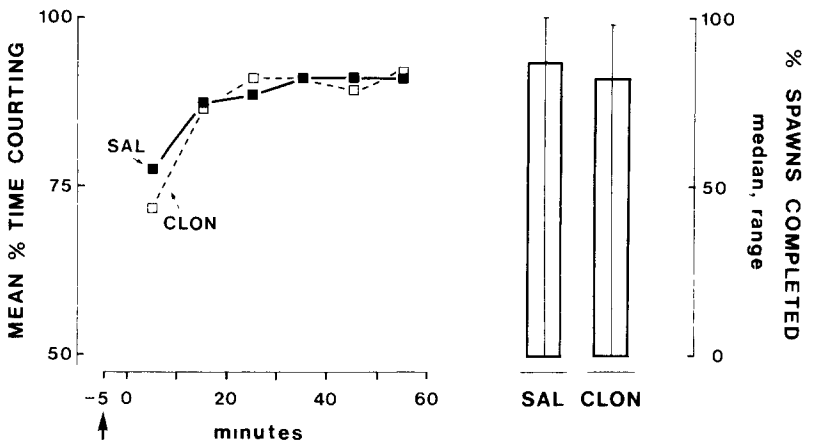

FIG 2 Lack of effect of clonidine (5 μg/g IP, arrow) on male spawning behavior in male goldfish Saline, n=11, clonidine, n=12

Behavioral Tests

Goldfish spawn by scattering adhesive eggs among the leaves and roots of aquatic vegetation; although both floating and benthic plants may be used as spawning substrates (personal observations), fish in this study were provided only with floating artificial plants Goldfish spawning behaviors include a persistent chasing or courtship of the female by the male(s) interspersed with a number of spawning acts, each of which is initiated when the female, accompanied by the male, rises into the aquatic vegetation [17]. In most spawning sessions, the pair occasionally emerges from the vegetation without performing a spawning act Female spawning behavior, which normally occurs only in ovulated females, can be readily induced in nonovulated females [20,25] and in males [21,24] by injection of PGF. Throughout the annual reproductive cycle, males will court and spawn with sexually active females, and females and males will perform female spawning behavior in response to PG injection.

With the exception that behavioral tests in this study were conducted in flow-through aquaria, testing procedures for female and male spawning behavior were basically as described previously [24] All tests were conducted between 1000 and 1500 hr (from 2–7 hr after the onset of photophase).

Statistics

Differences among groups for total female spawning acts in Experiment 1 were analyzed by Kruskal-Wallis analysis of variance and a nonparametric multiple paired comparisons test [7]. Differences between group scores for total female spawning acts in Experiments 2 and 3, and for duration of male courtship and percentage of completed spawning acts in Experiment 2 were analyzed by Mann-Whitney U-test. Two-tailed probabilities have been used in all cases.

EXPERIMENT 1

Following a pilot experiment in which clonidine (1 μg/g IP) significantly suppressed the spawning response to PGF (50 ng/g) in female goldfish, this experiment examined the dose-response of clonidine inhibition.

Method

Female goldfish were removed from the holding aquaria and injected with saline or 0 1, 0 5, or 2 5 μg/g clonidine (n=12 in all groups), followed immediately by PGF (50 ng/g). Within 2 min of the PG injection, fish were placed individually in observation aquaria with one sexually active stud male and observed continuously for the following 90 min.

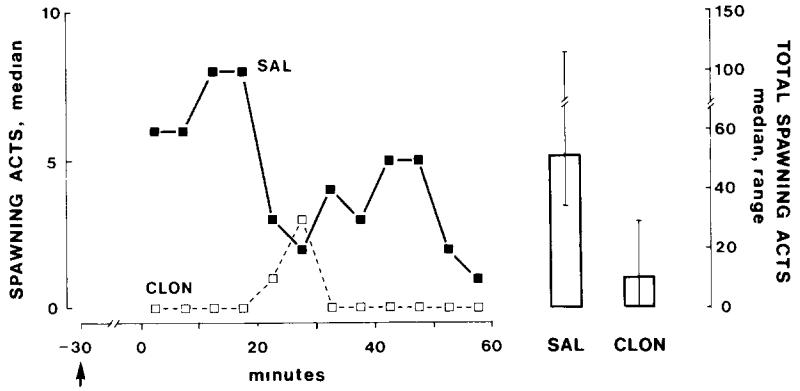

FIG 3 Inhibition of female spawning behavior in ovulated goldfish injected with clonidine (2.5 μg IP) 30 min before behavioral testing (arrow) N=5 in both groups.

Results and Discussion

The spawning response to PGF was significantly suppressed ($p<0.02$) by the high dose (2.5 μg/g) of clonidine, but unaffected by the medium or low dose (Fig. 1). Clonidine-treated fish showed no ill effects of the treatment and were courted vigorously by the stud males regardless of their level of spawning activity.

EXPERIMENT 2

This experiment was carried out to determine whether the inhibitory action of clonidine was restricted to female behavior, or whether male sexual behavior also might be affected.

Method

The experiment consisted of two parts. The first, which examined the effect of clonidine on female spawning behavior in males, was similar to Experiment 1 except that only saline (n=12) and one clonidine dose (2.5 μg/g; n=12) were used, and the behavioral tests were only 60 min in duration. In the second part, which examined the effect of clonidine on male behavior, males were removed from holding aquaria, injected with either saline (n=11) or clonidine (5 μg/g; n=12), and placed individually in observation aquaria which contained one PGF-treated female which had exhibited spawning behavior immediately prior to the test. Commencing 5 min after introduction of the test male, the duration of male courtship (chasing and following) and the number of incomplete spawning acts (entries of the female into the vegetation, with or without the male, which did not lead to a spawning act) and complete spawning acts were recorded continuously for the following 60 min.

Results and Discussion

Clonidine (2.5 μg/g) significantly inhibited ($p<0.02$) PG-induced female spawning behavior in male goldfish. Female spawning acts were performed by 8 of the 11 males injected with saline and PGF (median=12; range, 0–146), whereas none of the 12 males injected with clonidine and PGF exhibited any female spawning behavior. In contrast to the inhibitory action on female behavior in males, clonidine, even though given at a higher dose (5 μg/g), failed to affect either the duration of male courtship or the percentage of female spawning attempts which resulted in a spawning act (Fig. 2). There also was no difference in the total number of spawning acts performed by the saline-treated (median=10; range, 0–81) and clonidine-treated groups (median=17; range, 0–63). The results indicate that inhibition of PG-induced female spawning behavior by clonidine cannot be attributed to a non-specific debilitating action of the drug

EXPERIMENT 3

It is presumed that female spawning behavior in goldfish is regulated by endogenous PG, released from the reproductive tract into the bloodstream in response to the presence of ovulated eggs, and acting within the brain [21, 23, 25]. This experiment sought further support for this hypothesis by examining the effect of clonidine on spawning of ovulated fish.

Method

Females were removed from holding aquaria on the morning of the day of spontaneous ovulation, 1–6 hr after the assumed time of follicular rupture [22], and placed individually in observation aquaria containing one sexually active stud male to determine that they would spawn normally After they had performed several spawning acts, the females were removed, injected with either clonidine (2.5 μg/g; n=5) or saline (n=5) and returned to the holding aquaria; 30 min after injection, females were again placed individually in the observation aquaria and observed continuously for the following 60 min. Immediately after the test, fish were anesthetized and the volume of eggs remaining in the reproductive tract determined by gently expressing the eggs into a plastic vial and drawing them into a syringe.

Results and Discussion

Although 4 of the 5 clonidine-treated females performed at least one spawning act during the 60 min test period, clonidine significantly ($p=0.008$) inhibited spawning activity (Fig 3). All clonidine-treated fish retained ovulated eggs (0.68±0.28 ml) at the completion of the tests. In contrast, eggs were found in only one of the 5 saline-treated fish (0.02 ml), and it was clear that spawning in 3 of the saline-treated fish had terminated prior to the end of the test. As the saline-treated females would have continued to spawn had

oviposition not been completed, the results provide a conservative estimate of the ability of clonidine to inhibit spawning behavior in ovulated goldfish

GENERAL DISCUSSION

Clonidine significantly inhibited both the spawning behavior normally displayed by ovulated goldfish, and the female spawning response to PGF injection in nonovulated females and in males. As clonidine did not affect male spawning behavior in males, even though administered at twice the dosage effective for inhibiting female behavior in both sexes, the effects of clonidine on female behavior in the goldfish do not appear to be simply the result of a non-specific inhibition of behavior.

Although exogenous PGs exert opposite effects on female sexual behaviors of goldfish (stimulatory) [20] and guinea pigs (inhibitory) [18], clonidine antagonizes the actions of PGs in both species. The demonstrations that clonidine inhibits spawning behavior in ovulated goldfish, and prevents the rapid post-copulatory abbrevation of estrus in the guinea pig [14], provide indirect support for the hypotheses that endogenous PG regulates female spawning behavior in goldfish [21,23] and mating-induced estrus termination in the guinea pig [15]. The findings that blood levels of PGF are elevated shortly after ovulation in goldfish [1] and brook trout, *Salvelinus fontinalis* [3,10], and after copulation in guinea pigs [15], also are consistent with these proposed roles of endogenous PG in female reproductive behaviors

There is considerable evidence that clonidine prevents abbreviation of estrus in mated and PG-treated guinea pigs by increasing alpha-adrenergic activity [14]. For example, inhibition of norepinephrine synthesis [19] or injection of an alpha-adrenergic antagonist [8] blocks receptivity induced by estrogen-progesterone treatment Clonidine restores receptivity of steroid-treated females in which norepinephrine synthesis has been blocked, and this facilitatory action of clonidine can be inhibited by the alpha-adrenergic antagonist, phenoxybenzamine [19]. In addition, the alpha-adrenergic antagonist, yohimbine, prevents the maintenance of post-copulatory receptivity normally induced by clonidine [14]. Together with information that PGs suppress adrenergic activity [28], these findings indicate that clonidine antagonizes the behavioral action of PGs by increasing alpha-adrenergic activity [15]

There is no evidence from behavioral studies that clonidine inhibits female spawning behavior by stimulating an adrenergic system However, there is some evidence from endocrine studies in goldfish that clonidine functions as an adrenergic agonist. Both norepinephrine and clonidine elevate serum gonadotropin levels in female goldfish with ovaries in early or mid-recrudescence, but not in females in late ovarian recrudescence [4,5]; the stimulatory action of norepinephrine on serum gonadotropin levels is inhibited by phentolamine, an alpha-blocker [5]. The available information on clonidine action in goldfish thus does not rule out the possibility that in goldfish, as has been suggested for the guinea pig [15], clonidine and PG may act on an adrenergic system, likely within the brain, to modulate female sexual behavior

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