

Gonadotropin Releasing Hormone (GnRH) Affects Precopulatory Behavior in Testosterone-Treated Geldings

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MCDONNELL, S. M., N. K. DIEHL, M. C. GARCIA AND R. M. KENNEY. *Gonadotropin releasing hormone (GnRH) affects precopulatory behavior in testosterone-treated geldings*. *PHYSIOL BEHAV* 45(1) 145-149, 1989.—Twelve Pony geldings with (n=6) and without (n=6) testosterone replacement (200 µg/kg testosterone propionate in oil, SC every 48 hours) received either gonadotropin releasing hormone (GnRH; 25 µg SC every 3 hours) or control treatment. Sexual behavior was recorded during 4-minute exposure to an estrous mare, 3 times weekly for 2 weeks before treatment, 3 weeks during treatment, and 3 weeks after treatment had been discontinued. The group receiving testosterone and GnRH (n=3) exhibited significantly greater flehmen response frequency and attention duration and significantly lower vocalization frequency and erection duration than the group receiving only testosterone (n=3). GnRH had no apparent effect on sexual behavior in geldings without testosterone replacement (n=3). These results support an hypothesis of testosterone-dependent, CNS-mediated effects of GnRH on precopulatory behavior in the stallion.

GnRH	Sexual behavior	Horse	Stallion	Flehmen	Erection
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IN addition to their endocrine-mediated behavioral effects, hypothalamic releasing hormones have been implicated in direct central nervous system (CNS) effects on behavior (5). Current evidence suggests that these peptides or related fragments have neurotransmitter or neuromodulatory activity in the brain which influences behavior that precedes or prepares the animal for corresponding pituitary-endocrine induced behavioral patterns. In several laboratory species there is considerable evidence supporting a direct CNS role of GnRH in control of precopulatory or copulatory behavior in both males and females (12).

When horses are given an intravenous injection of gonadotropin releasing hormone (GnRH) in order to evaluate their pituitary and gonadal function, they often immediately exhibit a flehmen response (authors' unpublished observations). The flehmen response (lip curl) is a male-typical element of precopulatory and elimination/markings behavioral sequences associated with oronasal contact with urine; feces, or vaginal secretions [see (10) for description]. The flehmen response is believed to facilitate vomeronasal organ access to nonvolatile pheromonal substances. Domestic stallions with spontaneously low sexual interest or response often display more pronounced sexual interest in an estrous mare within a few minutes to 2 hours following administration of GnRH (authors' observations). Although the apparent effect on behavior seems more rapid than would be expected as a result of GnRH-induced rise in circulating androgens, steroid mediation of these behavioral effects has not been ruled out. The present experiment was conducted to evaluate direct CNS versus steroid-mediated effects of

GnRH on sexual behavior in the horse. Sexual behavior of castrated male horses (geldings) with and without testosterone replacement was measured before, during and after a 3-week period of chronic pulsatile subcutaneous administration of GnRH or saline control treatment. In this work, GnRH was given in a pulsatile fashion to approximate a hypothesized episodic pattern of endogenous GnRH release.

METHOD

Subjects

Twelve mixed-breed pony geldings (150 to 300 kg b.wt.) were acquired from auction or private farms. These ponies ranged in age from 3 to 20 years and had been castrated for at least 3 months before the start of the study. Precastration breeding history was not available. Circulating testosterone levels confirmed complete castration (<0.1 ng/ml by radioimmunoassay, New Bolton Center Clinical Endocrine Service). All animals were housed in one barn in individual stalls, and were allowed individual paddock exercise 3 times weekly. The animals were maintained on hay and grain fed twice daily and water provided ad lib. The study was conducted during June and July, which are natural breeding season months for ponies and horses in the northern hemisphere.

Treatments

Results of baseline sexual behavior trials were used to rank subjects from high to low precopulatory response level and to assign subjects to rank-matched sets for one of four

treatment regimens ($n=3$ per treatment): testosterone replacement (T), GnRH (GnRH), testosterone replacement plus GnRH (T-GnRH), and vehicles only control (C). Testosterone replacement consisted of 200 $\mu\text{g}/\text{kg}$ b.wt. testosterone propionate crystalline (Sigma Chemical Company, St. Louis, MO) dissolved in sesame oil, administered subcutaneously every 48 hours for 3 weeks in order to maintain plasma testosterone concentrations within the normal range for adult stallions (0.35 to 1.75 ng/ml). Weekly plasma samples were obtained by jugular venipuncture to monitor circulating testosterone. The GnRH treatment consisted of 25 μg GnRH (Cystorelin, CEVA Laboratories, Overland Park, KS) administered subcutaneously every 3 hours (0300, 0600, 0900, 1200, 1500, 1800, 2100 and 2400 hours) for 3 weeks in order to provide a pulsatile stimulus pattern. Based on clinical experience and preliminary observations in pony stallions, subcutaneous injection of 25 μg of GnRH results in an approximate doubling of plasma testosterone within one to two hours, with return to baseline within four to six hours. The schedule was designed to approximate an hypothesized endogenous pulsatile release of GnRH. Control treatment consisted of vehicle injections on the above schedules.

Sexual Behavior Trials

Sexual behavior was evaluated in standard trials conducted 3 times weekly for the 2 weeks prior to treatment (baseline period), 3 weeks during treatment (treatment period), and the 3 weeks following cessation of treatment (posttreatment period). Trials were conducted on a Monday-Wednesday-Friday schedule between 0730 hr and 0900 hr, which was 90 to 180 minutes following the 0600 hour injection of GnRH. Each trial consisted of a 4-minute exposure to an estrous pony mare which was restrained along the outside of a small outdoor enclosure (3.05x3.05 m, 1.5 m high post and rail fencing) in which the subject was confined. This arrangement permitted interaction through the rails and mounting of the fence, but prevented intromission. During such exposure, referred to as "teasing," in the horse industry, stallions and mares usually display most elements of the precopulatory sequence typical of free-running or pasture breeding horses (10). Two ovariectomized, estrogen-treated pony mares (estradiol cypionate 0.5 mg subcutaneously every other day) were used as stimulus mares. For each day of trials, each mare was used in randomly selected order for six consecutive trials. On each day, trial order of the twelve gelding subjects was also random. A hand-held microcomputer event recorder (Observational Systems, Inc., Seattle, WA) was used to measure the following sexual behavior responses: sniff mare (nose within approximately 10 cm of the mare), sniff ground (usually voided mare urine or feces), flehmen response, vocalization, penis drop, erection, and mount fence. Attention to the mare, defined as standing within close proximity (approximately 1 meter), was also recorded.

Statistical Analyses

Behavioral endpoints for each trial included frequency of each response, as well as penis drop latency and duration, erection latency and duration, mount latency and duration, and attention latency and duration. Latencies represented the time in seconds from the beginning of trial to the first occurrence of a response. Durations represented the total time engaged in a particular response. Data were analyzed

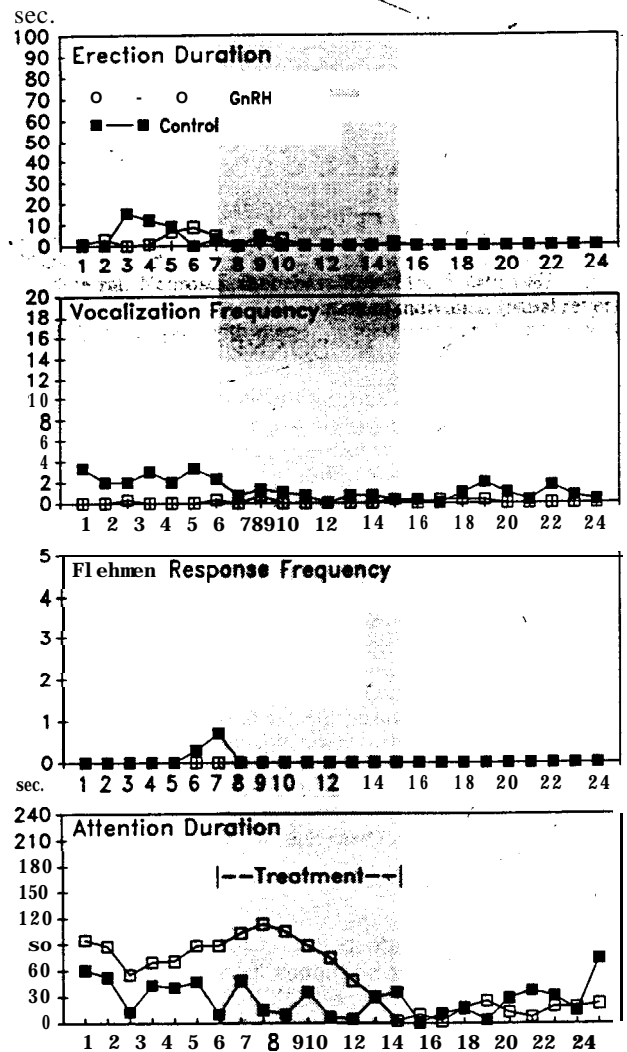


FIG. 1. Mean attention duration, flehmen response frequency, vocalization frequency and erection duration of GnRH- ($n=3$) and control- ($n=3$) treated ponies during 6 baseline trials (1-6), 9 treatment trials (7-15), and 9 posttreatment trials (16-24).

by repeated measures analysis of variance with 4 levels of treatment and 24 levels of trials. Fisher's protected least significant difference (LSD) procedure ($p<0.01$) was used for mean comparisons (19).

RESULTS

Plasma testosterone levels of testosterone-treated subjects increased during the first week of treatment to within the range for normal stallions (0.35 to 1.75 ng/ml). Levels remained within this range throughout treatment and returned to baseline concentrations (<0.1 ng/ml) within one week after treatment had ceased. Testosterone levels of control and GnRH only subjects remained within the normal range for geldings (<0.1 ng/ml) throughout the study.

There was a significant ($p<0.01$) group by trial interaction for sniff ground frequency, flehmen response frequency, at-

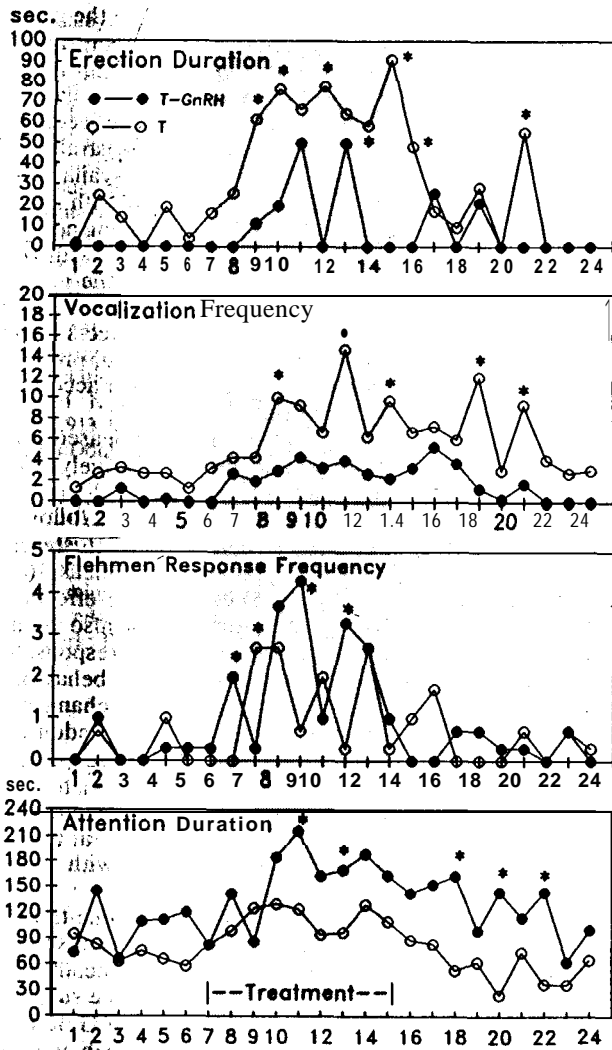


FIG. 2. Mean attention duration, flehmen response frequency, vocalization frequency, and erection duration of testosterone and GnRH- (T-GnRH $n=3$) and testosterone- (T, $n=3$) treated ponies during 6 baseline trials (1-6), 9 treatment trials (7-15), and 9 posttreatment trials (M-24). Asterisks indicate trials during which the group means are significantly different (LSD, $p < 0.01$).

tention duration, erection duration, vocalization frequency, penis drop latency, and penis drop duration. Further comparison of means using LSD ($p < 0.01$) revealed several apparent patterns of differences. During the baseline period, there were no patterns of significant differences among groups for any of these seven endpoints. GnRH and control treatment had no apparent influence ($p > 0.01$) on any of the sexual behavior measures. As shown in Fig. 1 for erection duration, vocalization frequency, flehmen response frequency, and attention duration, all measures for these two groups that did not receive testosterone suggested a gradual decline in sexual arousal and response over the course of the experiment.

Sexual behavior in all testosterone-treated animals was restored to within the range of intact stallions, but at the lower end of the range in terms of overall precopulatory

vigor for pony stallions (11). Generally, T and T-GnRH means (as illustrated for attention duration, flehmen response frequency, vocalization frequency and erection duration, Fig. 2) were significantly greater than those for baseline and were significantly different from those of GnRH and control animals during treatment. When testosterone treatment was discontinued, sexual behavior of these animals decreased toward pretreatment levels.

Comparison of T-GnRH and T groups indicated that for attention duration, flehmen response frequency, erection duration and vocalization frequency there were four or more trials after the onset of treatment during which the two groups differed significantly. The T-GnRH and T group means for these endpoints are shown in Fig. 2. For attention duration, for trials 11, 13, 18, 20, and 22, the T-GnRH mean was greater than the mean for the T group ($p < 0.01$). During the period of trials 11 through 24, the remaining means followed a similar pattern (T-GnRH apparently greater than T), although the differences did not meet the conservative 0.01 level of probability. For flehmen response frequency, T-GnRH means were significantly greater than means for T during trials 8, 11, and 13, while during trial 9, the T group showed greater ($p < 0.01$) flehmen response frequency than the T-GnRH group. For erection duration, for trials 9, 10, 12, 14, 15, 16, and 21, the T-GnRH mean was lower ($p < 0.01$) than the T-mean. For the period of trials 7 through 16, the remaining means followed a similar pattern of differences (T-GnRH less than T), although not consistently significant differences. For vocalization frequency, for trials 10, 13, 15, 19, and 21, the T group mean was significantly greater than the T-GnRH mean. For sniff ground frequency, penis drop latency, and penis drop duration, T-GnRH and T group means were significantly different only on 3 or fewer trials after the onset of treatment (not shown).

DISCUSSION

These results suggest a testosterone-dependent facilitatory effect of GnRH on attention to the mare and flehmen response in horses. These results are consistent with our earlier observations of GnRH-induced flehmen response in stallions, as well as with our clinical observations of several stallions with spontaneously low sexual interest and arousal which exhibited heightened interest in mares immediately following injection of GnRH. Attention time, while defined in terms of time spent near the mare, encompasses a period during which the animals typically engage in a number of different precopulatory responses, including sniff mare, sniff ground, lick, nuzzle, nip, and flehmen response. The occurrence and pattern of each of these specific precopulatory responses in horse seem to be peculiar to the individual (10). When studying a small number of subjects, differences in such specific responses may not be readily detectable, and attention duration serves as a reasonable estimate of precopulatory interest which reflects a sum of individual precopulatory responses.

In this study, animals receiving GnRH and testosterone exhibited significantly lower erection duration than those receiving only testosterone. This is in contrast to findings in male rats, where erection and mount latencies are decreased by GnRH treatment (13). One explanation for this decreased erection duration in our GnRH-treated ponies may be that, as a result of increased precopulatory investigation and flehmen response, the GnRH-treated animals did not

proceed as rapidly to copulatory responses. The observer recording the sexual behavior in this study subjectively described these animals as "fixated" on sniffing and flehmen response. The T-GnRH subjects' erection duration was lower than that of T subjects during sexual behavior trials, however the T-GnRH animals achieved erection and masturbated while standing quietly in the stall with what appeared to be greater frequency than T animals. Interpretation of differences between our results and those of studies in laboratory species is complicated by many factors, including important differences in the copulatory patterns, differences in the behavioral responses measured, and corresponding differences in the focus on arousal or potency aspects of sexual behavior. For example, in the majority of studies addressing behavioral effects of GnRH using laboratory species, olfactory investigatory behaviors have not been specifically studied. Vocalization frequency was also decreased by GnRH treatment in testosterone-replaced geldings. As with erection duration, lower response as a result of GnRH treatment is difficult to interpret. Effects of GnRH on vocalization apparently have not been evaluated in other species.

The absence of effects of GnRH on sexual behavior in geldings without testosterone replacement is consistent with previous observations that steroid priming is necessary for GnRH effects on sexual behavior. In castrated male rats (3, 14, 17) and voles (I), testosterone priming is necessary for GnRH effects on sexual behavior. Similarly, in female rats, estrogen priming is required for GnRH effect on lordosis behavior (15, 16, 18). In this regard, it is interesting that in our study the differences in attention duration, erection duration, and vocalization frequency persisted for about two weeks after treatment had been discontinued, which is typically the same pattern of decline of sexual behavior following castration (authors' unpublished observations under comparable conditions). We believe that this further supports the concept of testosterone-dependency of GnRH effects on sexual behavior.

While it is well established that peripheral administration of brain peptides induces behavioral effects similar to those induced by intracranial administration, there is scant evidence that these peptides actually cross the blood brain barrier and little is understood about how they exert CNS effects. Entry, distribution, and fate of peripherally administered GnRH in the CNS of horses apparently have not been studied. In other species, peripherally administered labeled peptides penetrate the blood brain barrier, though only in minute amounts (8). Electrophysiological changes have been detected at several brain sites following peripheral administration of peptides, including GnRH (8).

There is evidence suggesting that effects of peripherally administered GnRH on olfactory-related behavior may be mediated peripherally. GnRH-containing neurons have been found in clear association with olfactory pathways through-

out the central and peripheral nervous system. In the golden hamster, a well defined GnRH-containing tract, arising from the olfactory tubercle and two olfactory bulb nuclei, forms of peripheral autonomic-like system of fibers, ganglia, and neurons within the subarachnoid space between the hemispheres before joining the olfactory nerves rostral to the olfactory bulb (6). In horses, the limited data available on GnRH production and distribution is consistent with that of other mammalian species studied. G&I-I-containing neurons have been located throughout the hypothalamus, with high concentrations in the rostral preoptic areas and the median eminence, as well as in the limbic by&m and the olfactory tubercle (2). Perhaps the olfactory behavioral effects of peripherally administered GnRH in this study and our previous observations were mediated at the level of main or accessory (vomeronasal) olfactory receptors.

In horse mares, pituitary gonadotropins are released in response to pulsatile intravenous injection of relatively small amounts of GnRH (2 to 20 µg) (7). In horse stallions subcutaneous injection of 25 µg of GnRH is typically followed within 1 to 2 hours by a 100% increase in plasma testosterone levels, presumably mediated by gonadotropin release (authors' unpublished observations). Therefore, the effects of GnRH treatment observed in this study might also be attributable to pituitary gonadotropins released in response to treatment. In female rats, GnRH-induced behavioral changes have been shown to be independent of changes in pituitary or gonadal hormone levels (16,18). In addition, fragments and synthetic analogs of the GnRH decapeptide which are not active in the pituitary have been shown to have behavioral activity similar to that of the entire decapeptide (4,9). Further, work in horse geldings indicates that testosterone propionate pretreatment is associated with a decreased LH reponse to GnRH treatment (20).

In summary, GnRH treatment in testosterone-treated geldings increased attention duration and flehmen response frequency and decreased erection duration and vocalization frequency. These findings provide further evidence supporting the hypothesis of an extraendocrine effect of GnRH on sexual behavior. GnRH did not affect sexual behavior of geldings without testosterone replacement, which is consistent with existing evidence that the effects of GnRH on behavior are testosterone dependent.

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