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Phencyclidine Sedation as a Technique for Handling Rhesus Monkeys: Effects on LH, GH, and Prolactin Secretion[1] (39227)

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(Introduced by M. R. Nocenti)

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A major problem in the experimental study of endocrine secretion in the rhesus monkey is the choice of a proper method of restraint. Endocrine systems may be markedly influenced by usual anesthetic or tranquilizing agents (1, 2). Anesthetic problems may be avoided by restraining the animals in primate chairs. These monkeys, however, are readily alarmed, and it may be difficult to obtain stable control levels for hormones which are easily influenced by stress.

The following report details results from experiments involving the secretion of LH, prolactin, and GH in primates immobilized with phencyclidine (Sernylan). This agent acts as a mild depressant to the central nervous system (3, 4). At low doses, it induces calmness and decreases aggressiveness so that handling of the animal during the experimentation period is greatly facilitated.

Materials and methods. Adult female rhesus monkeys, weighing 4.0–5.0 kg were housed in an air-conditioned room with a controlled 12-hr light-dark schedule. The presence of menstrual bleeding was checked daily, and only animals with regular menstrual cycles were selected for these experiments. The effects of tranquilization with phencyclidine (1-{1-phencyclohexyl} piperidine) hydrochloride (Sernylan, Bioceut labs) were studied in the following experimental procedures and were compared to results obtained in unsedated animals.

(i) Daily LH levels throughout a menstrual cycle were measured in 7 animals, in whom blood collection by femoral vein puncture was facilitated by daily sedation with 5 to 10 mg of phencyclidine given im between 9 and 10 AM, 15 to 30 min before blood was drawn. These results were compared to those obtained from seven animals from whom blood samples were drawn without sedation by using a squeeze cage. Progesterone was assayed in at least two samples collected during the luteal phase in each animal as a check that ovulation had occurred.

(ii) In eight ovariectomized monkeys, pulsatile LH secretion and the inhibitory effect of estradiol-17β on LH levels were compared during both sedation and restraint. All animals had been ovariectomized at least 5 months previously. For sedation, the monkeys received 5 to 10 mg ($\sim$1.5 mg/kg) of phencyclidine at the beginning of the 8-hr experiment, and the injections were repeated as needed, generally at approximately 2-hr intervals. The animals were placed on an electrically warmed surface, and the body temperature, recorded rectally, was kept at normal by adjusting the heat. Restrained animals were placed in primate chairs 16–18 hr prior to the experiments. In both groups, blood samples were collected every 20 min from a catheter in the femoral vein. Estradiol-17β, 800 ng, was injected intravenously after a 4-hr control period. The estrogen was dissolved in absolute alcohol and diluted in physiological saline to 1% alcohol concentration.

(iii) LH release following subcutaneous

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administration of estradiol benzoate (250 μg in oil), on Day 3 of the menstrual cycle, was studied in 7 sedated and 10 restrained animals. The animals were restrained in a primate chair or sedated as in the preceding experiment. Blood samples were collected every 2 hr for a period of approximately 52 hr.

(iv) Levels of GH and prolactin under sedation and restraint were compared in four monkeys during the early follicular phase. Catheterization of the femoral vein was performed in all animals at 8:30 AM. Blood samples were collected every 20 min for 5 hr, starting at 12 Noon, to avoid the possible effects of the cannulation stress (5). Phencyclidine sedation was maintained as needed. During the experiment, the usual activity in the laboratory went on, except for the telephone and machinery which were disconnected.

(v) Prolactin and GH response to synthetic TRH were studied in five sedated and seven restrained monkeys. Fifty micrograms of TRH were injected iv. Blood samples were collected at frequent intervals during 4 hr following injection. Sedation and cannulation were performed as described in the previous experiment.

LH levels were measured by a heterologous radioimmunoassay using antibovine LH serum (6) and LH-LER 1213 as a standard. This assay was described previously (7). GH and prolactin levels were estimated by radioimmunoassay in terms of human standards (8, 9). Progesterone was measured by a competitive protein binding assay (10).

Results. Effects on the menstrual cycle. Sedation after a single injection of phencyclidine (~1.5 mg/kg) lasted from 1 to 2.5 hr. All animals showed an LH surge whether the blood sample was taken under sedation or not (Fig. 1). Mean LH values during the cycle were not statistically different in the two groups. Progesterone measured during the latter part of the cycle (5–12 ng/ml) indicated that ovulation had occurred in all sedated and unsedated animals. Menstrual cycle lengths during the experimental cycle ranged from 26 to 34 days in animals receiving phencyclidine daily and from 26 to 29 days in unsedated animals. These values

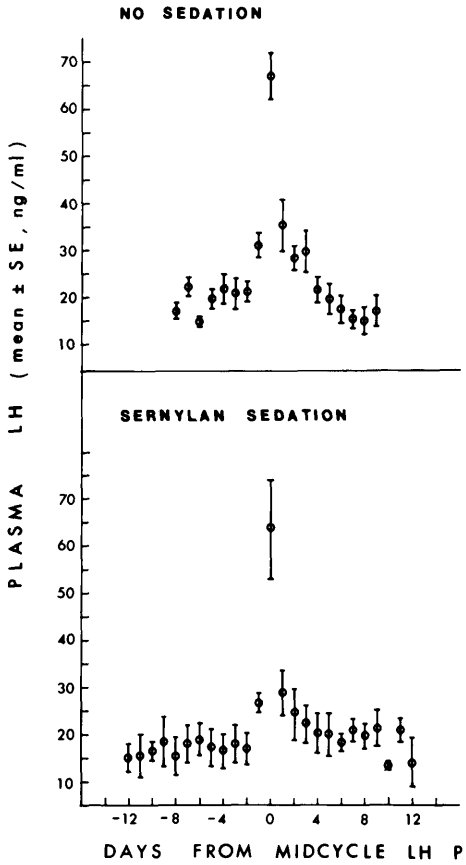

FIG. 1. Daily plasma LH levels throughout the menstrual cycle in seven unsedated rhesus monkeys and in seven animals sedated with phencyclidine.

were not significantly different from those seen in the preceding and following cycles.

Two animals had longer cycles; one sedated monkey had a 53-day cycle, and one unrestrained monkey had a 41-day cycle.

Effects on LH secretion in ovariectomized animals. LH secretion was pulsatile in all eight ovariectomized animals, whether under continuous phencyclidine sedation or restraint. The frequency of the pulses (60–150 min) varied from animal to animal but was apparently not altered by sedation. Intravenous injection of 800 ng of estradiol-17β depressed LH levels in both groups (Fig. 2). Depression in LH levels occurred rapidly in most cases. However, in a few individuals, in both groups, an additional pulse of LH occurred before the response to estradiol was seen. Low LH levels persisted for a minimum of 4 hr in both groups.

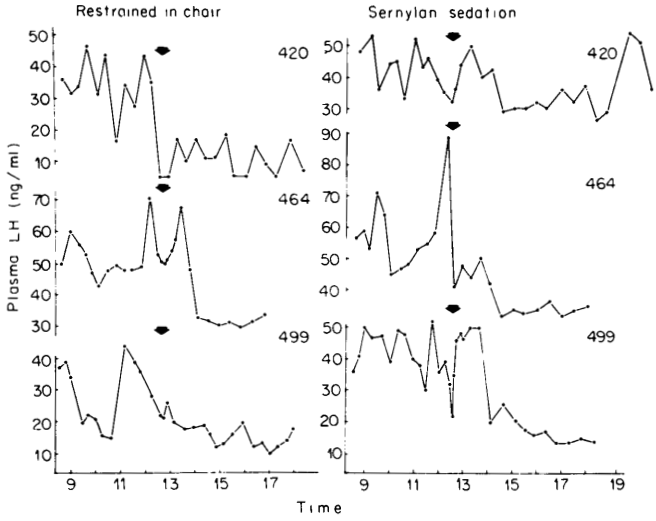

FIG. 2. Plasma LH levels in three ovariectomized rhesus monkeys restrained in a primate chair or under phencyclidine sedation for a period of 8–10 hr. Blood samples were collected at 20-min intervals. The arrow indicates the time of iv injection of 800 ng of estradiol-17β.

Effects on estrogen-induced LH surge. Estradiol benzoate, injected on Day 3 of the menstrual cycle, induced an LH surge in all 10 restrained animals. Immediately following estrogen administration, LH levels declined and remained low for approximately 24 hr, after which they increased to reach peak values 38 to 42 hr after the estrogen injection. Peak levels reached were similar to those seen during the midcycle LH surge (Fig. 3).

Only two of the seven animals under continuous sedation had an LH surge following estrogen injection.

Effects on GH and prolactin levels. During the 5-hr experiment, stable levels of GH were seen in $^2/_4$ sedated and $^1/_3$ restrained animals (Fig. 4). In the other animals, one or two secretory peaks were observed. Stable levels of prolactin were seen in $^2/_4$ sedated and $^2/_3$ restrained animals. The secretory episodes of GH and prolactin did not coincide.

Effects on prolactin and GH response to TRH. TRH induced a release of prolactin in all sedated and restrained monkeys (Fig. 5). While the time-course of release was similar in both groups, the amount of prolactin released after TRH was significantly greater in the phencyclidine treated animals ($P < 0.05$–<0.01). GH levels remained unchanged after TRH, except in one unsedated monkey where they increased significantly.

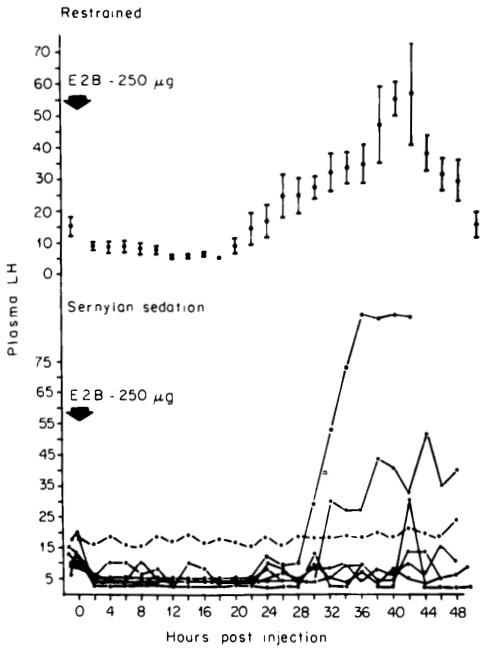

FIG. 3. Plasma LH levels after S.C. estradiol benzoate (E_2B) on Day 3 of the menstrual cycle in 10 monkeys restrained in a primate chair (mean ± SE) or in seven monkeys under phencyclidine sedation for a period of 52 hr.

Discussion. The anesthetic effects of phencyclidine are markedly different from

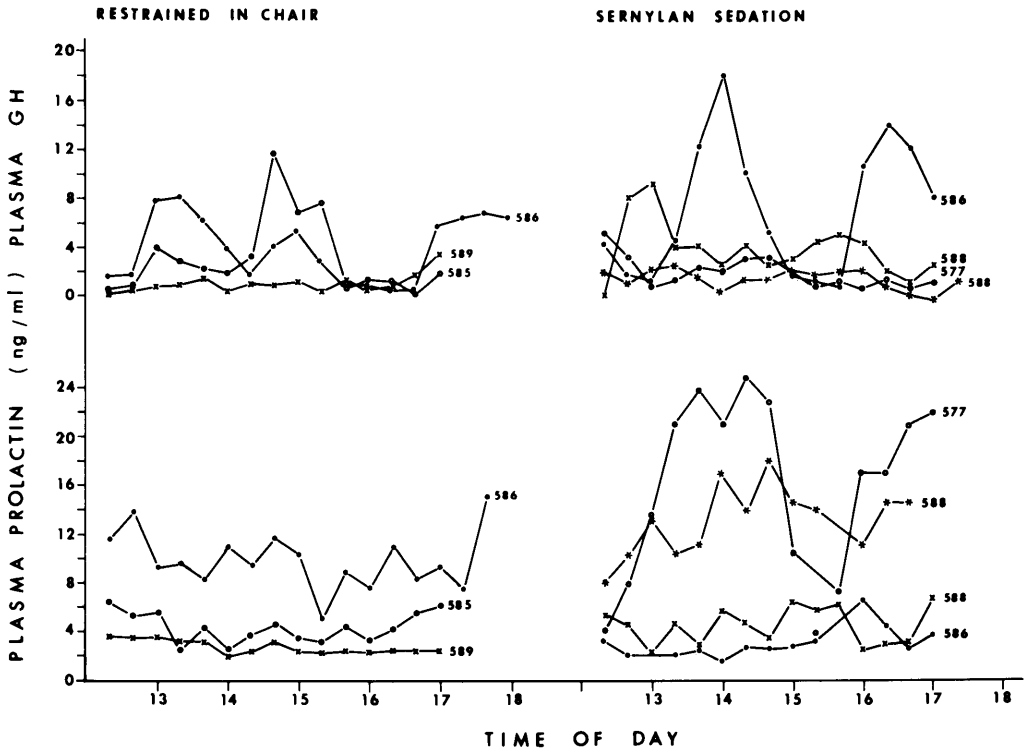

FIG. 4. Plasma GH and prolactin levels during a 5-hr period in four monkeys restrained in a primate chair or under phencyclidine sedation. Sedation was started at 8:30 AM.

those of the usual anesthetic agents. The animals are quieted, but most brain stem and somatic reflexes are maintained, muscle tone is preserved, and respiration is not affected. Tranquilization is accompanied by hypersalivation. The dose of phencyclidine used (1.5 mg/kg) induces tranquilization within 5 min and the effects last for 1 to 2.5 hr. Excess dosage results in increased muscular tonicity with spasms, which can easily be confused with signs of inadequate dosage. With experience, such problems do not occur.

Phencyclidine tranquilization greatly facilitates experimental manipulation of rhesus monkeys. Our results indicate that this method may be used in the study of certain aspects of endocrine secretion. A single daily injection of phencyclidine, which sedates the animals for only a short period of time, can be used to facilitate blood sample collection without interfering with the menstrual cycle ovulatory process. Prolonged sedation, for up to 8 hr, does not interfere with the pulsatile mode of release, which has been shown to exist for most pituitary hormones in monkeys as well as humans (11–16). LH secretion remains frankly pulsatile in sedated ovariectomized monkeys and is not qualitatively different from that seen in restrained animals. As in unsedated monkeys (17), estradiol-17β in sedated animals exerts a rapid inhibitory action on LH and its pulsatile mode of release. As for GH secretion, it is of interest to note that spurts of GH occur in sedated animals observed for a certain period of time. This phenomenon has also been reported in unsedated monkeys (16, 17). Since phencyclidine may not block all sensory impulses, such fluctuations in GH secretion may be related to external stimuli. However, no apparent relationship was found between environment stimuli and the spurts of GH. Episodic release of prolactin occurred in both restrained and sedated monkeys independent of any fluctuation in GH. In an earlier paper, we have shown that both insulin and 2-deoxy-glucose can cause an increase in GH secretion and a rise in plasma cortisol in sedated monkeys (18).

It is not known if phencyclidine affects

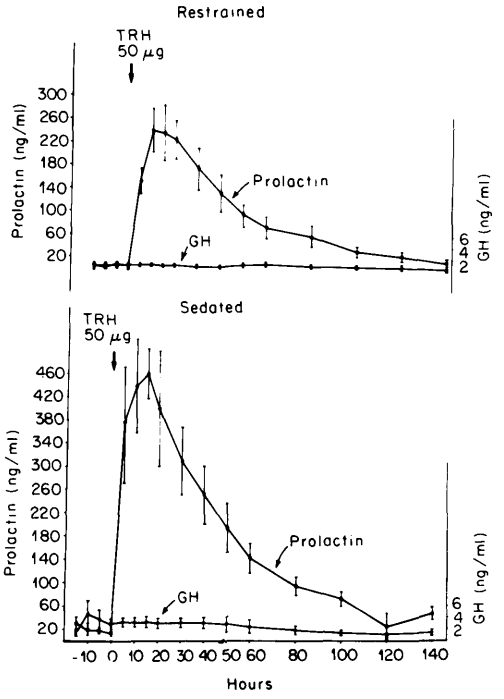

FIG. 5. Prolactin and GH response to an iv injection of TRH in six monkeys restrained in a primate chair and six monkeys under sedation. (mean ± SE).

neurohormonal or brain monoamine metabolism as do certain other tranquilizers (19). From our results, however, certain effects of prolonged phencyclidine sedation on LH or prolactin secretion may be explained by such a mechanism. Under prolonged phencyclidine sedation, prolactin response to TRH is significantly enhanced. While a short-term daily sedation does not interfere with the midcycle ovulatory LH surge, long-term sedation with phencyclidine prevents, in some monkeys, the LH surge that follows the administration of estradiol in the early follicular phase. Since phencyclidine does not prevent the release of LH after Gn-RH (7) or of prolactin after TRH, the inhibitory step probably occurs at a level above the pituitary. In the rat, various central depressants, such as barbiturates, chlorpromazine, or reserpine, also inhibit the spontaneous LH surge and ovulation when given during the critical period on the afternoon of proestrus (1). By contrast, in the rhesus monkey, phenobarbital has been reported to have no inhibitory effect on LH release after estrogen administration (20).

Summary. Rhesus monkeys, sedated with phencyclidine hydrochloride (Sernylan), were quieted for prolonged periods of time, while maintaining somatic reflexes, muscle tone, and respiration. Brief daily periods of sedation did not interfere with the menstrual cycle. Prolonged sedation, however, interfered with the experimentally estrogen-induced LH surge, but not with the inhibitory action of estrogen on LH tonic secretion. Pulsatile release of LH, GH, and prolactin persisted even under prolonged sedation. The secretion of prolactin in response to the administration of TRH was increased in animals sedated with phencyclidine.

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