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Failure of Daily Injections of Ketamine HCl to Adversely Alter Menstrual Cycle Length, Blood Estrogen, and Progesterone Levels in the Rhesus Monkey[1] (39862)

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Introduction. There has been a suspicion that the stress of restraining rhesus monkeys for the purpose of taking daily blood samples may alter the menstrual cycle length as well as ovarian hormone secretion rates. However, investigators have been hesitant to use any agents for restraint since there is always the suspicion that they might interfere with normal ovarian as well as pituitary and hypothalamic function. The present studies were therefore designed to examine the effects of ketamine, a mild anesthetic agent, upon menstrual cycle length, blood estrogen, and progesterone levels during several sequential menstrual cycle of rhesus monkeys given daily injections of ketamine HCl.

Materials and Methods. Thirty adult female rhesus monkeys (*Maccaca mulatta*) weighing between 4.2 and 6.5 kg were used, for a period of 4 years. Each monkey was observed daily for menses and cared for essentially as outlined before (1). Menstrual data during the months of July and August were not included in these experiments since it was found by us that menstrual flow diminished and became more irregular at this time. During the 25 control cycles, no ketamine was administered. Daily 5-ml blood samples were taken by saphenous puncture for 25 menstrual cycles over a period of 12 months. In 13 of these instances blood samples were taken from Day 5 to Day 20 of the menstrual cycle, and in 12 of these instances blood samples were taken from Day 1 to the end of the menstrual cycle. The monkeys were restrained by a squeeze-back cage, and one leg was exposed to enable a blood sample to be taken. In the experimental cycles ketamine HCl (8–10 mg/kg; Bristol-Myers) was administered 2–4 min prior to taking daily 5-ml femoral puncture blood samples. In the experimental cycles blood samples were taken from Day 5 to Day 20 in 13 instances, and in 19 instances blood samples were taken from Day 1 to the end of the menstrual cycle. Blood samples were taken using a 20-gauge needle and a 6-ml syringe. The blood samples were allowed to clot over night in the refrigerator (4°) and centrifuged for 20 min at 1000*g*. The serum was removed and frozen at −20° until assayed later for estrogen and progesterone.

Serum estrogen was extracted from 0.2-ml serum aliquots with 2.5 ml of diethyl ether and assayed in duplicate by radioimmunoassay using the antiserum against estradiol 17β succinyl-BSA as described originally by Hotchkiss *et al.* (2) and validated by us (3, 4).

Serum progesterone was extracted from 0.1-ml serum aliquots with 2.5 ml of petroleum ether and assayed in duplicate by the radioimmunoassay originally outlined by Thorneycroft and Stone (5) and modified by Karsh *et al.* (6) and validated by us (3, 4).

In some experiments ovarian vein samples were taken on the day of the preovulatory estrogen surge according to methods outlined by us (3).

An ovulatory cycle was defined as one in which the monkey had a normal preovulatory estrogen peak which was greater than 200 pg/ml accompanied by a characteristic midluteal-phase progesterone level of 3–6 ng/ml. In some instances these criteria were met accompanied by luteal-phase progesterone levels which were not consistently elevated; these cycles have been referred to as "low-progesterone luteal-phase" cycles. Statistical comparisons were carried out using a Student's *t* test.

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Results. As shown in Table I, the average menstrual cycle length of the cycles observed in the ketamine-treated versus control animals did not differ ($P > 0.5$). Administration of 8–10 mg/kg of ketamine HCl did, however, reduce the incidence of vaginal bleeding which occurred about midway throughout the luteal phase of the cycle which was being subjected to daily bleedings. It is believed that these early menses were brought about by the stress of the blood sampling procedure, which was more pronounced in the control compared to the ketamine-treated group. The incidence of ovulatory cycles was greater (88%) in the ketamine-treated cycles versus the control cycles (56%).

As shown in Figs. 1 and 2, the serum estrogen and progesterone levels throughout the menstrual cycle in the control and ketamine-treated monkeys were essentially similar. There was a follicular-phase rise and a preovulatory peak of estrogen followed by a sharp fall and relatively low

TABLE I. EFFECT OF DAILY ADMINISTRATION OF KETAMINE HCl ON MENSTRUAL CYCLE LENGTH AND OCCURRENCE OF OVULATORY MENSTRUAL CYCLES.[a]

	Observation	Monkey treatment	
		Control	Ketamine
(A)	Menstrual cycles during which monkeys were sampled for blood		
	Average menstrual cycle length in days (ave ± SE)	30 ± 1.6	28 ± 0.9
	Number of menstrual cycles observed	70	88
(B)	Menstrual cycles of monkeys whose blood samples were subjected to serum progesterone and estrogen estimations		
	Number of menstrual cycles examined	25	32
	Instances where monkeys menstruated early during the luteal phase	5	1
	Incidence (%)	20	3
	Ovulatory cycles (number)	14	28
	Incidence (%)	56	88
	Cycles exhibiting low luteal-phase progesterone levels (%)	30	25
	Anovulatory cycles (%)	44	22

[a] A sample of 30 rhesus monkeys was observed over a 4-year period. Animals were either given ketamine HCl (8–10 mg/kg) or no treatment prior to taking each blood sample. The monkeys were subjected to daily blood sampling from Days 6 to 20 or from Day 1 until the end of the cycle.

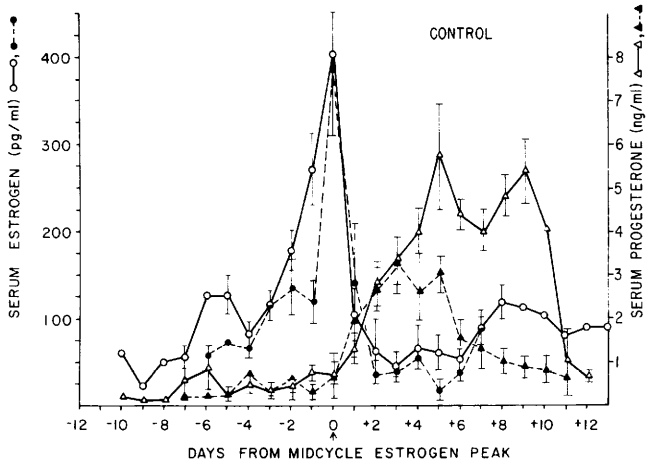

FIG. 1. Serum estrogen and progesterone levels throughout the menstrual cycle of 12 normal control monkeys (shown by solid lines connecting open circles and triangles) and of 4 control monkeys exhibiting "low luteal-phase" progesterone levels (shown by dashed lines connecting solid circles and triangles). The data have been normalized according to the day of the midcycle estrogen peak in order to correct for differences between lengths of menstrual cycle of individual monkeys. In the case of the normal controls, values of estrogen and progesterone represent the mean ± SEM of 12 observations from Days 6–8 and of 2 to 6 observations from Days −10 to −7 and from Days +9 to +13.

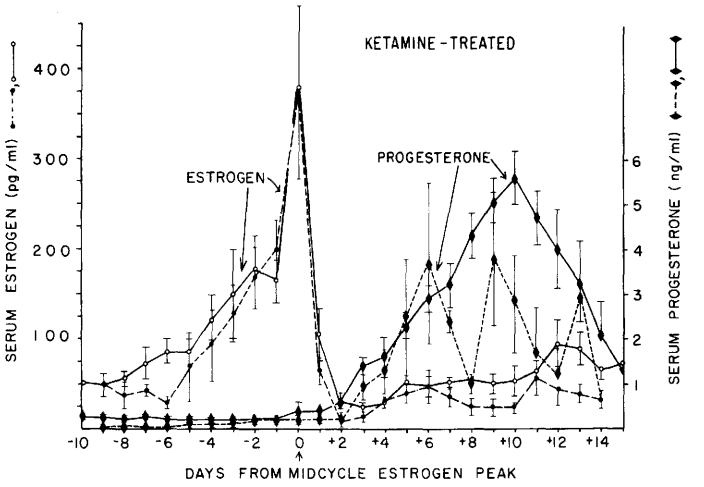

FIG. 2. Serum estrogen (open circles connected by solid lines) and progesterone (solid diamonds connected to solid lines) levels throughout the menstrual cycle of 15 monkeys given injections of 8–10 mg/kg of ketamine HCl immediately prior to taking each blood sample. Data were normalized according to the day of the midcycle estrogen peak. Values represent the mean ± SEM of 8 to 20 observations. Estrogen (solid circles connected by dashed lines) and progesterone (solid diamonds connected by dashed lines) levels of five ketamine-treated monkeys exhibiting a low-progesterone luteal phase are also shown.

luteal-phase estrogen level with a rise occurring during the latter half of the luteal phase. The progesterone levels during the luteal phase of the control and ketamine-treated monkeys were consistent with ovulatory cycles and remained elevated with a maximum occurring about 10 days after the midcycle estrogen peak. In about 25% of the monkeys given ketamine HCl (5/19) and in 30% of control animals (3/10) there were luteal phases in which progesterone levels were lower than normal ($P = 0.02$) and in which estrogen levels were normal (Figs. 1 and 2). In these cycles luteal-phase progesterone levels started to rise until 5–8 days after the estrogen surge, at which time they fell and rose slightly and then fell again at the end of the luteal phase of the cycle. In these instances, the length of the cycle was normal. There also were anovulatory cycles in both the control (44%) and the ketamine-treated (22%) animals which were characterized by a consistently low level of estrogen and progesterone (Tables I and II). Ovarian vein estrogen levels on the day of the preovulatory estrogen peak of control and ketamine-treated animals were essentially similar, ranging from 2.5 to 7.5 ng/ml (Table II).

TABLE II. ESTROGEN LEVELS IN PERIPHERAL SERUM AND IN OVARIAN VENOUS PLASMA OF CONTROL AND KETAMINE-TREATED MONKEYS.

	Monkey treatment	
Serum sample	Control	Ketamine
Peripheral serum on day of preovulatory estrogen peak	355 ± 30[a] (n = 12)	283 ± 40 (n = 20)
Midcycle serum sample taken in anovulatory monkeys	72 ± 7 (n = 18)	43.5 ± 9.4 (n = 6)
Ovarian vein sample taken from ovary containing preovulatory follicle	3072 ± 510 (n = 9)	5944 ± 2034 (n = 5)

[a]Estrogen levels in picograms per milliliter (mean ± SEM).

Discussion. It is apparent that, within this small sample of rhesus monkeys, daily use of 8–10 mg/kg of ketamine HCl did not significantly alter the length of the menstrual cycle nor did it lead to significant alterations in estrogen or progesterone levels throughout the menstrual cycle. Furthermore, use of ketamine lead to an increase in ovulatory cycles (88%) compared to control cycles (56%). It is probable that the higher incidence of anovulatory cycles as well as the incidence of premature menses observed in the control animals were due to the

stresses of handling the monkeys for the purpose of obtaining blood samples. The possibility does exist, however, that had the control animals been more acclimated to the restraint methods used, their incidence of anovulatory cycles might have been lower. The daily serum estrogen and progesterone levels observed in the control and ketamine-treated animals resembled those observed in untreated animals by Hotchkiss *et al.* (2), Weick *et al.* (8), and Neill *et al.* (9).

Our observance of cycles which consisted of a normal preovulatory estrogen peak and initiation of a normal luteal-phase progesterone level up until 5–8 days after the estrogen surge followed by a decline in progesterone and observance of variable low luteal-phase progesterone levels until the end of the luteal phase could be accounted for by the presence of a low progesterone-secreting corpus luteum. This situation was observed to a similar extent in control monkeys (30%; 3/10) compared to ketamine-treated monkeys (25%; 5/15). The causal factors for this situation are not known. Others have not observed a low progesterone level during the normal-duration luteal phase in the rhesus monkey. Observations of a shortened luteal phase plus low luteal-phase progesterone levels have, however, been made by Sherman and Korneman (10) and by Strott *et al.* (11) in the human and by Wilks *et al.* (12) in the monkey. Sherman and Strott and their colleagues have attributed the "short luteal phase" to a decreased FSH:LH ratio in the preovulatory gonadotropin surge (10, 11). We cannot explain the etiology of the low-progesterone luteal phase observed in our monkeys. Since gonadotropin measurements were not carried out in our studies we cannot evaluate LH and FSH contribution to the low-progesterone luteal phase observed. The levels of estrogen in the preovulatory estrogen peak in the monkeys exhibiting a low-progesterone luteal phase were similar to those in monkeys having normal luteal-phase progesterone levels. Therefore, the size of the estrogen surge cannot be used as a predictor of whether or not a given monkey will have an adequate luteal phase later on during the menstrual cycle. This was also true in the case of the short luteal phase observed by Wilks *et al.* (12). It could be that the factors leading to the low-progesterone luteal phase may not be related to the preovulatory estrogen peak and may be related to other factors. Whether or not follicular rupture occurred in these particular cycles was not ascertained. It is possible that this represents luteinization of an atretic follicle. These possibilities are currently under investigation.

Ferin *et al.* (13) have used phencyclidine (Sernylan) as a restraining agent and have found that it also does not alter menstrual cycle length or LH levels in female rhesus monkeys. Both types of agents act as dissociative anesthetic agents (14) with a hypersalivatory effect. However, in our experience the hypersalivation complication has been less pronounced with ketamine compared to Sernylan.

Summary. This study was carried out using 30 adult female rhesus monkeys in order to determine the effects of daily administration of ketamine HCl (8–10 mg/kg) upon menstrual cycle length, incidence of ovulatory menstrual cycles, and blood estrogen and progesterone levels throughout the cycle. In physically restrained control monkeys (25 cycles subjected to daily blood sampling) there were 14 of 25 or 56% ovulatory cycles, and in ketamine-treated monkeys (32 menstrual cycles subjected to daily blood sampling) there were 28 of 32 or 88% ovulatory cycles. The length of the menstrual cycle was the same in both groups. The levels and time course of peripheral serum estrogen and progesterone levels were the same in the ovulatory cycles of both groups. In some of the control cycles (30%) and in some of the ketamine-treated cycles (25%) there were luteal phases in which the preovulatory estrogen levels were normal and in which the luteal-phase progesterone levels were low and variable 6–8 days after the preovulatory surge. It can be concluded that the daily use of ketamine HCl does not significantly alter menstrual cycle length, or serum estrogen or progesterone levels throughout the menstrual cycle. It reduced the incidence of anovulatory cycles and premature menstrual induction

probably by reducing the stress of restraining the monkey for the purpose of taking a blood sample.

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