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Alteration of Pulsatile LH Secretion in Monkeys by Pentobarbital Anesthesia (41274)

WILLIAM B. WEHRENBERG AND MICHEL FERIN

Center for Reproductive Sciences, and the Departments of Obstetrics and Gynecology and Physiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Abstract. The effects of deep barbiturate anesthesia on pulsatile LH secretion were determined in nine ovariectomized macaque monkeys (*Macaca nemestrina* and *Macaca mulatta*). To allow each monkey to serve as its own control, gonadotropin secretion was evaluated twice in each one, once while conscious and once while anesthetized. Anesthesia was induced by the injection of 60–120 mg sodium pentobarbital (iv) followed by the continuous infusion (12 hr) of sodium pentobarbital at 15 mg/hr. Blood samples were drawn every 30 min for the duration of the experiment (12 hr). The frequency of LH pulses in conscious monkeys (5.4 ± 0.7 pulses/12 hr) was significantly greater ($P < 0.001$) than that in anesthetized monkeys (2.8 ± 0.2 pulses/12 hr). Pulse characteristics were also different between the two groups. In conscious monkeys LH pulses peaked in one 30-min sample and then gradually declined over the next two to five samples. In contrast, LH pulses were sustained over two to four 30-min samples and then declined over the next four to six samples in anesthetized monkeys. These results clearly demonstrate that deep barbiturate anesthesia has a pronounced effect on pulsatile LH release in monkeys and suggest that general central nervous system depression has an inhibitory effect on LH secretion.

The interruption of normal LH secretion by sodium pentobarbital in rats is well established (1). Barbiturate anesthesia has been utilized in numerous species to evaluate the feedback mechanisms which regulate spontaneous or steroid-induced LH release (2–4). In contrast to these unequivocal effects of sodium pentobarbital on gonadotropin secretion in rodents, this drug has been reported to have no effect on LH secretion in monkeys (5, 6).

Our observations that anesthesia can block the estradiol-induced LH surge in monkeys (7) and that the frequency of pulsatile GnRH secretion in anesthetized monkeys (8) is less than the frequency of pulsatile LH secretion in conscious ones (9) has led us to question the reported lack of effect of sodium pentobarbital on gonadotropin secretion in monkeys. To elucidate the possible effects of deep barbiturate anesthesia on LH secretion, we have evaluated pulsatile LH secretion in conscious and anesthetized ovariectomized monkeys.

Materials and Methods. Seven female pigtailed monkeys (*Macaca nemestrina*) and two female rhesus monkeys (*Macaca mulatta*) weighing 4.5 to 6.0 kg were utilized in this experiment. All animals had been bilaterally oophorectomized at least 2 months prior to the experiment. Animals were housed individually in a temperature-controlled environment (19–22°) under a 12:12 light:dark schedule. The monkeys were fed monkey chow daily and their diet was supplemented three times per week with fresh fruit.

Each animal was utilized twice, once conscious and once anesthetized in random order, and thereby served as its own control. The protocol for the experiment in the conscious monkeys was to place them into a primate restraining chair under light sedation (ketamine–HCl, 20–30 mg) on the evening prior to the experiment and move them into the laboratory. The next morning between 0700 and 0800 hr a femoral vein catheter was inserted percutaneously and the animals were then left undisturbed for the duration of the experiment. The protocol for the experiment in the anesthetized monkeys was to lightly sedate them (as above) on the morning of the experiment and move them into the laboratory. A femoral vein catheter was then inserted, followed by the administration of sodium

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pentobarbital (60 to 120 mg, iv) to induce stage 3, plane 1 to 2, anesthesia (10). This is characterized by deep regular breathing, abolishment of eyelid reflex, and eccentric eyeballs which may or may not oscillate. This plane of anesthesia was then maintained for the duration of the experiment by a constant infusion of sodium pentobarbital at a rate of 15 mg/hr. If this rate of administration failed to maintain this plane of anesthesia, the monkey received an additional injection of barbiturate (15 to 30 mg), and the infusion rate was increased to 20 to 30 mg/hr. After the effects of ketamine sedation had worn off (approximately 1 hr after insertion of the catheter), blood sampling began. Samples (1.5 ml) were drawn every 30 min for 12 hr, and an equal volume of normal saline was injected into the animal following each sample. Samples were allowed to clot overnight at 4°. Serum was collected and stored at −20°.

LH concentrations were determined by radioimmunoassay (11) using monkey gonadotropin preparation LER-1909-2 as the reference standard. Two of the monkeys (Pt-16 and Pt-18), although demonstrating pulsatile LH secretion, had abnormally low LH concentrations. For this reason LH was also determined by the interstitial cell–testosterone bioassay system in these two monkeys. The method of Neill *et al.* (12) was followed except that a mouse interstitial cell preparation was used to avoid the collagenase step. The same reference standard as in the radioimmunoassay was used. Although LH concentrations were higher in these two animals using the bioassay, the pattern of LH release was identical regardless of the assay system utilized. Radioimmunoassay results are used to express the LH concentrations except for monkeys Pt-16 and Pt-18 where bioassay values are used. Within- and between-assay variations were respectively 5 and 17% for the radioimmunoassay and 8 and 18% for the bioassay.

LH pulses were characterized by at least a 100% increase in LH concentrations over the value obtained from the preceding one or two samples. In some instances, the LH pulses increased only approximately 25 to 100%; however, their identification was unequivocal. Statistical differences were determined by analysis of variance. All data are expressed as mean ± SEM.

Results. Deep sodium pentobarbital anesthesia had a pronounced inhibitory effect on LH pulse frequency and altered LH pulse characteristics. Illustrative examples are presented for two monkeys in Fig. 1 and the data are summarized in Table I. Since there was no significant difference in mean LH concentrations and frequency of LH pulses between species and assay methods, the data were pooled. In two animals (Pt-9 and Pt-16) the LH pulse frequency was similar in the conscious and anesthetized state. However, the pulse frequency in these conscious monkeys was much lower than that in the other conscious animals. In the remaining seven monkeys there was a marked reduction in the number of LH pulses during anesthesia. Overall, the pulse frequency per 12 hr was significantly greater ($P < 0.001$) when the monkeys were conscious (5.4 ± 0.7) than when they were anesthetized (2.8 ± 0.2). The LH pulse characteristics in conscious monkeys were to peak in one 30-min sample and then gradually decline over the next two to five samples. In marked contrast, the characteristics in anesthetized monkeys were to

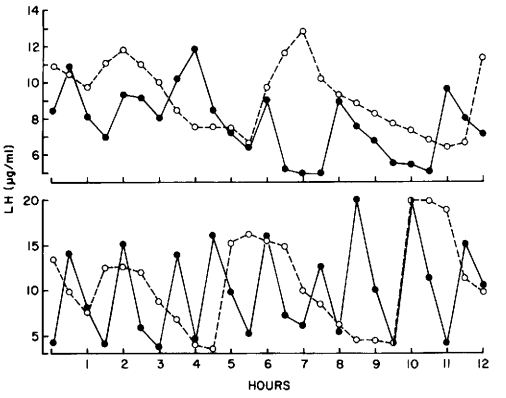

FIG. 1. Pulsatile LH secretion in two ovariectomized monkeys (upper plot, rhesus-659; lower plot, pigtailed-19). The solid lines (●——●) represent the pattern while the monkeys were conscious and the dashed (○- - -○) represent the pattern while the monkeys were anesthetized.

TABLE I. FREQUENCY OF LH PULSES AND MEAN LH CONCENTRATION IN CONSCIOUS AND ANESTHETIZED PIGTAILED (Pt) AND RHESUS (Rh) MONKEYS

	Conscious		Anesthetized	
Monkey No.	Pulses/12 hr	Mean LH (μg/ml)	Pulses/12 hr	Mean LH (μg/ml)
Pt-9	3	23.3 ± 1.9	3	26.4 ± 2.3
Pt-16	2	9.4 ± 1.5	2	13.1 ± 2.0
Pt-10	5	14.2 ± 1.3	2	11.4 ± 1.6
Pt-12	5	17.9 ± 1.9	2	16.0 ± 1.9
Pt-18	6	10.1 ± 2.6	3	12.5 ± 2.5
Pt-19	9	9.8 ± 1.0	4	10.8 ± 1.0
Pt-20	6	8.3 ± 0.9	3	13.4 ± 0.8
Rh 639	7	7.5 ± 0.2	3	9.0 ± 0.2
Rh 659	6	7.7 ± 0.4	3	9.2 ± 0.4
	5.4 ± 0.7[a]	12.0 ± 1.8	2.8 ± 0.2	13.5 ± 1.8

[a] Mean ± SEM, significantly different ($P < 0.001$) from pulses/12 hr in anesthetized monkeys.

peak over two to four 30-min samples followed by a gradual decline over the next four to six samples. As a result of this sustained elevation of the LH pulses in the anesthetized monkeys, the mean concentration over the 12-hr period was not different between the conscious (12.0 ± 1.8 μg/ml) and anesthetized (13.5 ± 1.8 μg/ml) animals.

Discussion. These results in macaque monkeys are the first report that sodium pentobarbital can cause an unequivocal suppression of pulsatile LH secretion in a subhuman primate. They are in contrast to the report by Bhattacharya *et al.* (5) which suggested that the administration of sodium pentobarbital had no or equivocal effect on the secretory pattern of LH in castrated monkeys. The mode of administration of the anesthesic agent in their study was either a single injection or an initial injection followed by other smaller injections over the next few hours. Although we brought our animals under anesthesia with an initial injection similar to that used by Bhattacharya *et al.* (5), we subsequently maintained them on a relatively constant plane of anesthesia by a chronic 12-hr infusion of sodium pentobarbital. The deep stage of anesthesia attained in our animals is the most likely explanation for the divergence in these two reports. Our demonstration that deep barbiturate anesthesia alters pulsatile LH secretion casts some doubt on the specificity of α-adrenergic blockers and neuroleptics in suppressing LH (5).

Pulsatile LH secretion is presumably a direct consequence of pulsatile GnRH secretion from the hypothalamus (8, 13). Cognizant of this, we believe that the altered LH pulse frequency in anesthetized monkeys is a result of altered GnRH secretion. If this is the case, what remains to be answered is whether sodium pentobarbital, or, for that matter, any other centrally acting agent, alters pulsatile GnRH by a direct effect on the GnRH neuron or by modulating other neuronal input on the GnRH neurons. However, Carter and Dyer (14) have reported that pentobarbital can suppress the LH response of the pituitary to GnRH *in vitro*. This suggests that the altered LH pulse frequency during anesthesia might also be due to an altered pituitary sensitivity to GnRH.

That barbiturate anesthesia may suppress GnRH secretion or alter pituitary sensitivity to it leads to the speculation that sodium pentobarbital may be able to block the midcycle gonadotropin surge in primates as is the case in rodents (see introduction). Supporting this possibility is the observation by Ferin *et al.* (7) that continuous phencyclidine sedation (52 hr) blocked the estrogen-induced LH surge in five of seven monkeys. In contrast, Knobil (6) reported that continuous pentobarbital anesthesia

(36 hr) did not block the estradiol-induced LH surge in three monkeys. Again this discrepancy may be ascribable to the stage of anesthesia.

The initial description of LH secretion in ovariectomized monkeys described the pulses as circhoral due to their approximate hourly occurrence (9). In our first attempts to monitor pulsatile GnRH secretion into the hypothalamic–hypophyseal portal blood in monkeys (8), we reported intervals between GnRH pulses as long as 3 to 4 hr. In retrospect, this discrepancy between the LH and GnRH pulse frequency may have been a direct effect of barbiturate anesthesia during the portal blood collection.

Even though the LH pulse frequency was greater in conscious monkeys, the mean LH concentration over the 12-hr period was similar in the two groups of animals as a result of altered pulse characteristics. The mechanism by which these pulses are altered by anesthesia is unknown. Possible explanations may include an increase in the size of the releasable pool of LH as a result of the longer interval between pulses or a change in LH metabolism under anesthesia. An alternative explanation of the fact that average LH values were not different would be the existence of a short feedback loop for LH (i.e., LH regulating LH concentrations). The existence of a short feedback loop for LH has been proposed in the human (15); however, experiments investigating this possibility in monkeys have been hampered by the unavailability of monkey LH (6).

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