PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175, 487–490 (1984)

Influence of Restraint and Ketamine Anesthesia on Adrenal Steroids, Progesterone, and Gonadotropins in Rhesus Monkeys (41825)

G. B. FULLER,* W. C. HOBSON,* F. I. REYES,† J. S. D. WINTER,‡ AND C. FAIMAN§

*Primate Research Institute, New Mexico State University, P.O. Box 1027, Holloman AFB, New Mexico 88330-1027, †Department of Obstetrics and Gynecology, State University of New York Downstate Medical Center, Brooklyn, New York 11203, and ‡Department of Pediatrics and §Departments of Medicine and Physiology, University of Manitoba, Winnipeg, Manitoba, Canada

Abstract. Changes in gonadotropins, progesterone, cortisol, DHA, and DHAS were monitored in 10 female rhesus monkeys (Days 20–23 of the menstrual cycle) subjected to cage restraint with or without ketamine anesthesia for successive venipunctures. All animals were bled without sedation for 2 hr at 30-min intervals. Then 4 of the animals were anesthetized with ketamine-HCl and bleedings in all animals were continued for an additional 2.5 hr. FSH and progesterone were not appreciably affected by either restraint technique. LH declined steadily for the duration of the bleedings ($P < 0.05$). Serum levels of cortisol and the adrenal androgens increased twofold ($P < 0.05$). Anesthesia with ketamine had no effect on any of the six variables when compared with saline controls. Cortisol and dehydroepiandrosterone (DHA) levels tended to plateau ($P < 0.01$) after 2 hr in both treated and control groups. In contrast, dehydroepiandrosterone sulfate (DHAS) levels increased continuously throughout the entire study period. These data indicate that ketamine anesthesia does not alter endocrine responses to venipuncture when administered following cage restraint of conscious animals. These findings further confirm the difficulties in obtaining estimates of basal levels of hormones which are responsive to stress and suggest that the first sample may provide the best estimate.

Determination of basal circulating hormone levels in nonhuman primates presents a problem since handling or restraint of the animal for venipuncture may introduce sufficient stress to change hormonal secretion. Primate chairs, manual restraint, and anesthesia are the most commonly used techniques for obtaining consecutive blood samples.

In rhesus monkeys both restraint (primate chair or squeeze cage) and anesthesia have been shown to affect adrenal and pituitary secretion (1), although the effects of sedation have been difficult to separate from the concomitant effects of handling. Interestingly, Castro *et al.* (2) reported that ketamine anesthesia did not alter basal plasma concentrations of insulin or cortisol in chair-adapted, chronically cannulated cynomologus monkeys, an observation which suggests that ketamine itself may not affect some endocrine functions under restraint conditions where animal handling is minimal. However, many experimental designs prohibit the use of chair restraint and cannulation, and it is not clear whether ketamine can alter the endocrine responses which normally accompany continuous acute restraint for venipuncture. Accordingly, the present study was designed to examine the effect of ketamine anesthesia on hormonal patterns when superimposed upon endocrine responses which had already been induced by squeeze cage restraint and venipuncture.

Materials and Methods. *Animals.* Ten mature, cycling female rhesus monkeys were used in this investigation which was initiated at the end of May and completed in August. This time interval corresponds to the reported summer anovulatory period in rhesus monkeys. The animals were housed individually in air-conditioned, day–night-regulated quarters. The light–dark cycle consisted of 12 hr light (beginning at 0600) and 12 hr dark. Standard Purina monkey chow was provided daily, supplemented with fresh fruit three times per week. Females with a history of normal menstrual cycles were selected for the investigation. Each animal was tested between Days 20 and 23 following menses with blood sampling conducted between 0830 and 1300 hr. Each bleeding was made in conscious monkeys after restraining the animal from 2 to 4 min with the squeeze mechanism of the cage. The arm was manipulated through an opening in the

0037-9727/84 $1.50

cage mesh and the blood sample (5–6 ml) taken by brachial venipuncture. All monkeys were bled alert at 30-min intervals for 2 hr beginning at 0830 hr (time 0). After the 2-hr bleeding, 4 animals received an im injection of ketamine HCl (Vetalar, Parke-Davis), 8 mg/kg body weight. Additional ketamine injections (5–10 mg/kg) were given as required to maintain complete sedation. Six control animals received injections of sterile saline of comparable volume immediately after the 2-hr bleeding. Intermittently restrained (as above), conscious and anesthetized monkeys were then bled at 30-min intervals for an additional 2.5 hr. Samples were centrifuged and the serum stored at −20°C until radioimmunoassays were done.

Hormone assays. Assays for rhesus FSH and LH were performed by previously described heterologous radioimmunoassay methods (3). The LER-M-907D assay standard was kindly provided by Dr. L. E. Reichert, Albany Medical College, and contained (by bioassay) 0.26 NIH-FSH-S1 U/mg and 0.025 NIH-LH-S1 U/mg. Radioimmunoassays for cortisol, dehydroepiandrosterone (DHA), dehydroepiandrosterone sulfate (DHAS), and progesterone were performed by previously described methods (4, 5).

All samples from an individual animal were assayed in a single assay run for each hormonal analysis. The intraassay coefficients of variation ranged from 5 to 8% and the interassay variability ranged from 10 to 14% for all samples.

Statistical analysis. Comparisons were made by analysis of variance using the BMD computer system, program P2V (6). This analysis recognized one independent factor (group; saline or ketamine) and one correlated factor (period; pre- vs post-treatment). The time data within the pre- and post-treatment periods were tested for linear trends. All main effects and interactions were tested for significance. In addition, the combined means at each time interval were compared with the first samples (time 0) mean value by Duncan's multiple-range test (7).

Results. *Gonadotropins.* Serum gonadotropin patterns were not altered by the administration of ketamine anesthesia following 2 hr after successive blood sampling of manually restrained monkeys (Fig. 1). Aside from an initial increment at 30 min ($P < 0.05$) mean

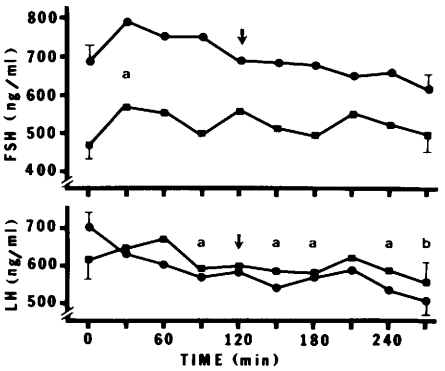

FIG. 1. Mean serum FSH and LH concentrations expressed in terms of the crude rhesus monkey pituitary standard LER-M-907D in two groups of rhesus monkeys. At times prior to the arrow, blood samples were obtained under intermittent manual (squeeze cage) restraint. Following the arrow, samples were obtained under ketamine anesthesia (■; $N = 4$ animals) or manual restraint (saline injections) (●; $N = 6$). The error bars at the beginning and end of each group's data set indicate the overall SEM for each point. Letters (a, $P < 0.05$, b, $P < 0.01$) above indicate statistical significance vs time 0 of the combined data from all 10 animals (see text for details).

FSH levels remained stable in both groups. Mean FSH and LH levels were not statistically different between the two groups throughout the study period. When serum LH values for both groups were combined, a steady decrease from time 0 was apparent with a significant time difference between pre- and post-treatment levels ($P < 0.01$). The overall decrement in mean levels was 21%.

Steroids. The effects of successive venipuncture and physical restraint on adrenal–ovarian steroid levels are illustrated in Figs. 2 and 3. Mean serum cortisol, DHA, and DHAS levels rose two- to threefold from the initial bleeding to the final sample at 270 min. The cortisol and DHA increments were statistically significant by 30 min ($P < 0.05$), whereas DHAS levels did not rise significantly until 90 min ($P < 0.05$). Although the slope of the concentration curve for cortisol and DHA tended to plateau ($P < 0.01$) in both ketamine and control groups after 2 hr, DHAS values continued to rise throughout the entire study period. Sedation with ketamine after 2 hr did not change the level or pattern of any of the three adrenal steroids when compared with the saline control.

Progesterone concentrations were not af-

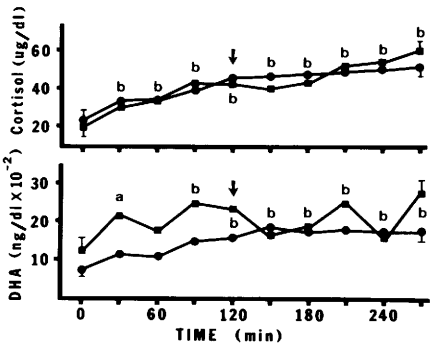

FIG. 2. Mean serum cortisol and dehydroepiandrosterone (DHA) concentrations in the two groups of rhesus monkeys. See legend to Fig. 1 for details.

fected by either anesthesia or repeated venipuncture although it should be noted that four of the animals in the saline treatment group showed nonluteal progesterone levels (<200 ng/dl).

Discussion. The progressive rise over a 2-hr period in circulating cortisol, DHA, and DHAS levels induced by repeated restraint and venipuncture were not altered by superimposing multiple injections of ketamine over a subsequent 2.5-hr sampling period. These results indicate that, under our acute experimental conditions, ketamine does not modify the stress-induced increase of either cortisol or adrenal androgens. These findings differ from those of Puri *et al.* (8) who used a similar experimental design but found that ketamine did cause an increase in cortisol levels above those in unanesthetized controls. No explanation is apparent for this disparity.

The plateauing of serum levels of cortisol and DHA after 2 hr contrasted with a progressive rise in values of DHAS throughout the 4.5-hr experimental period. These findings suggest that the repeated restraint and venipuncture induced a near maximal adrenal response associated with continuing peripheral conversion of DHA to DHAS. The much slower clearance of DHAS relative to DHA and cortisol (9) might also help to explain this difference in patterns.

The varying restraint techniques utilized for collecting consecutive blood samples from rhesus monkeys have led to somewhat different conclusions regarding the effects of stress and/or anesthesia on hormonal measurements. Whether an animal is restrained in a squeeze cage or a primate chair, or is anesthetized, appears to make little difference to the adrenal response as reflected by cortisol levels (8, 10, 11). Indeed, cortisol increased even in monkeys trained to offer a limb for venipuncture (10), although the mean cortisol level is significantly less in trained than in untrained animals. Rhesus monkeys maintained for extended periods under ketamine or phencyclidine anesthesia show rising cortisol concentrations with time (8, 10, 11), but this effect appears to be reduced or absent in chronically cannulated monkeys (2).

Restraint or anesthetic immobilization for venipuncture does not influence all endocrine parameters as serum concentrations of FSH and progesterone did not appear to be affected (1, 8). Channing *et al.* (12) have reported that daily injections of ketamine did not alter menstrual cycle length or circulating estrogen and progesterone levels in rhesus monkeys. However, under the present 4.5-hr sampling protocol, serum LH did show a modest (21%) decline with time, a finding which was not observed following shorter periods of cage restraint (8), ketamine (8), or phencyclidine tranquilization (1). In spite of the small decrease with time in serum LH, the restraint procedure used for certain reproductive studies would not be as critical a variable as in studies utilizing adrenal steroids as an endpoint.

These results confirm and extend previous reports of an effect of handling upon circulating cortisol levels and further demonstrate that ketamine anesthesia does not influence a previously induced stress response. In ad-

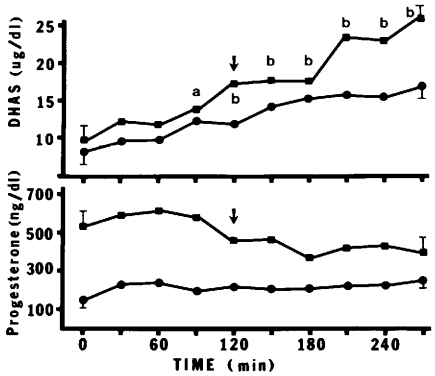

FIG. 3. Mean serum dehydroepiandrosterone sulfate (DHAS) and progesterone levels in two groups of rhesus monkeys. See legend to Fig. 1 for details.

dition, adrenal androgens also increase following cage restraint and consecutive venipuncture. Progesterone and FSH levels were generally unaffected by handling or anesthesia, whereas LH declined with successive sampling. These data substantiate the experimental problems in assessing basal levels of serum hormones. Based on our results, it appears that the initial sample would more closely represent the basal concentration in the blood.

The authors thank Mr. Scott Murray of the Primate Research Institute for excellent technical assistance and Ms. Cindy J. Wong, Computer Department for Health Sciences, Faculty of Medicine, University of Manitoba for helpful statistical advice.

1. Ferin M, Carmel PW, Warren MP, Himsworth RL, Frantz AG. Phencyclidine sedation as a technique for handling rhesus monkeys: Effects on LH, GH, and prolactin secretion. Proc Soc Exp Biol Med **151**:428–433, 1976.
2. Castro MI, Rose J, Green N, Petersen D, Taub D. Ketamine-HCl as a suitable anesthetic for endocrine, metabolic, and cardiovascular studies in *Macaca fascicularis* monkeys. Proc Soc Exp Biol Med **168**:389–394, 1981.
3. Faiman C, Stearns EL, Winter JSD, Reyes FI, Hobson WC. Radioimmunoassay for rhesus monkey gonadotropins. Proc Soc Exp Biol Med **149**:670–676, 1975.
4. Carter JN, Tyson JE, Warne GL, McNeilly AS, Faiman C, Friesen HG. Adrenocortical function in hyperprolactonemic women. J Clin Endocrinol Metab **45**:973–980, 1977.
5. Blankstein J, Faiman C, Reyes FI, Schroeder ML, Winter JSD. Adult-onset familial adrenal 21-hydroxylase deficiency. Amer J Med **68**:441–448, 1980.
6. Dixon WJ (ed). Biomedical Computer Program. Univ Berkeley, California Press, 1981.
7. Steel RGD, Torrie JH. Principles and Procedures of Statistics. New York, McGraw-Hill, pp107–109, 1960.
8. Puri CP, Puri V, Anand Kumar TC. Serum levels of testosterone, cortisol, prolactin and bioactive luteinizing hormone in adult male rhesus monkeys following cage-restraint or anesthetizing with ketamine hydrochloride. Acta Endocrinol **97**:118–124, 1981.
9. Beaulieu EE, Corpechot C, Dray F, Emiliozyi R, Lebeau MC, Mauvais-Jarvis P, Robel P. An adrenal-secreted "androgen": Dehydroisoandrosterone sulfate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates in man. In: Pincus G, ed. Recent Progress in Hormone Research. Vol 21:pp411–494, 1965.
10. Elvidge H, Challis JRG, Robinson JS, Roper C, Thorburn GD. Influence of handling and sedation on plasma cortisol in rhesus monkeys (*Macaca mulatta*). J Endocrinol **70**:325–326, 1976.
11. Wickings EJ, Nieschlag E. Pituitary response to LRH and TRH stimulation and peripheral steroid hormones in conscious and anesthetized adult male rhesus monkeys (*Macaca mulatta*). Acta Endocrinol **93**:287–293, 1980.
12. Channing CP, Fowler S, Engel B, Vitek K. Failure of daily injections of ketamine HCl to adversely alter menstrual cycle length, blood estrogen, and progesterone levels in the rhesus monkey. Proc Soc Exp Biol Med **155**:615–619, 1977.

Received October 14, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted December 28, 1983.