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Ketamine–HCl as a Suitable Anesthetic for Endocrine, Metabolic, and Cardiovascular Studies in *Macaca fascicularis* Monkeys (41292)

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Abstract. Ketamine–HCl has been reported, depending on experimental conditions and dosage given, to have significant cardiovascular and endocrine effects in some species. However, previous studies in primates have inadequately distinguished between animal handling and ketamine effects. We, therefore, examined the effects of various doses of ketamine (0, 5, 10, 15, and 20 mg/kg) on mean arterial blood pressure and on plasma insulin, glucose, and cortisol concentrations in 10 chronically cannulated *Macaca fascicularis* monkeys which had been acclimated to restraining chairs. Each monkey received three different doses of ketamine according to a balanced incomplete block design. Ketamine anesthesia produced no significant changes in plasma insulin, glucose, or cortisol concentrations nor did it affect mean arterial blood pressure. In addition, the effects of ketamine–HCl on endocrine responses to insulin-induced hypoglycemia were examined in four animals according to a cross-over design. These animals also had chronically maintained cannulas and had been acclimated to restraining chairs. Plasma glucose concentrations, as well as plasma ACTH, growth hormone, and cortisol responses in ketamine-anesthetized animals receiving an insulin challenge were no different from those in unanesthetized control animals. Thus, our studies indicate that ketamine–HCl does not perturb these particular hormonal systems in *M. fascicularis* monkeys.

Ketamine–HCl is an anesthetic that is widely used in veterinary medicine. Its short-acting properties make it particularly desirable in the routine handling of primates. Various studies, however, seem to indicate that ketamine may have significant cardiovascular and/or endocrine effects in both primate and nonprimate species (1–6). Many of these studies, however, fail to separate effects due to animal handling from those due to ketamine. The present studies were designed to examine the effects that ketamine has in *Macaca fascicularis* monkeys on some endocrine and cardiovascular parameters under conditions in which the effects of animal handling are tightly controlled. In particular, the effects of ketamine on basal levels of plasma glucose, cortisol, and insulin as well as hormonal responsiveness to insulin-induced hypoglycemia were evaluated. The use of chronically cannulated animals which were adapted to restraining chairs allowed for minimal animal handling during these studies and thus for a more accurate representation of any effects that ketamine may have.

Methods. Adult male *M. fascicularis* monkeys were gradually adapted to chair restraint for approximately 2 weeks. This was accomplished by placing the animals in restraining chairs for short periods of time initially and then increasing the length of time the animals spent in chairs as the animals became accustomed to chair restraint. Animals were considered to be fully adapted to chair restraint when they showed no physiological signs of stress or discomfort upon chair restriction for a period of 2–3 days.

At this time, food and water were withheld for 24 and 6 hr, respectively, prior to the surgical implantation of arterial and venous cannulas. Anesthesia was induced

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with 20 mg/kg ketamine (Ketalar, Parke-Davis, Detroit, Mich.) and was maintained with subsequent doses of ketamine as needed. Polyethylene catheters were inserted into a femoral artery and vein and advanced to the descending aorta and inferior vena cava. The hindlimb fascia and muscle incisions were sutured, and the catheters were tunneled through subcutaneous fascia to exit on the midback of the monkey. The hindlimb skin was then sutured. The catheters were tucked in a restraining jacket (Alice P. Chatham; Los Angeles, Calif.), and the animals were returned to their chairs. Penicillin (Squibb, Penicillin G, approx 100,000 units) was administered daily for 5 days postsurgery. Heparinized saline was routinely placed in the cannulas in order to maintain the catheters patent. A 2- to 3-day recovery period from surgery elapsed before any experimental procedures were begun.

The effects of five different doses of ketamine in isotonic saline (0, 5, 10, 15, and 20 mg/kg) on plasma levels of cortisol, insulin, and glucose were studied with a balanced incomplete block design (7). This experimental design allowed the random assignment of three out of the five doses to each of 10 monkeys. The order in which each animal received the three treatments assigned to it was also randomized. At least 24 hr elapsed between administration of successive doses.

Blood pressure and heart rate were monitored continuously during the experimental procedure by connecting the arterial cannulas to a Brush recorder. Mean blood pressure was calculated by adding one-third of the pulse pressure to the diastolic pressure. Blood samples (3.0 ml) were collected in lightly heparinized syringes from the venous cannulas at 5 min before and at 5, 10, 30, 60, and 90 min after an injection of ketamine into a thigh muscle. A constant blood volume was maintained by infusion of saline (3.0 ml) after collection of each blood sample.

Blood samples were spun on a table-top centrifuge for 10 min, and the plasma samples thus obtained stored at $-4°$ until the time of the assay. Plasma aliquots were assayed for insulin, cortisol, and glucose. Plasma insulin was measured by a commercial radioimmunoassay kit (Amersham, Arlington Heights, Ill.); intraassay and interassay coefficients of variation were <12 and $<20\%$, respectively. Plasma cortisol was also measured by radioimmunoassay (8); coefficients of variation of intra- and interassay variability were <8 and $<12\%$, respectively. The intra- and interassay variability was <8 and $<12\%$ for the ACTH assay and <5 and $<12\%$ for the growth hormone (GH) assay. Plasma glucose was measured by a glucose-oxidase enzymatic assay (9). Intraassay and interassay variations were <3 and $<11\%$, respectively.

The effect of ketamine on the hormonal responses to insulin-induced hypoglycemia was studied in an additional four animals. These animals were also chair-adapted and had undergone surgical implantation of arterial and venous cannulas, as mentioned previously. The monkeys were randomly assigned to two groups: the first group received ketamine anesthesia during the first experimental session, whereas the second group received ketamine anesthesia during the second experimental session according to a cross-over design. A 2-day period elapsed between experimental sessions. Ketamine anesthesia was induced by intramuscular injection of 15 mg/kg ketamine and maintained by 10-mg injections every 30 min. Regular, crystalline insulin (0.10 unit/kg, given im) was administered to all animals ten min after an im injection of ketamine or saline. Blood samples were obtained before insulin injection and at 15, 30, 60, and 90 min after injection. Plasma concentrations of cortisol, GH, and ACTH were measured by radioimmunoassay (8, 10, 11). Blood glucose was measured as described previously.

Statistical analysis of the effect of different ketamine doses on basal endocrine status was done with analysis of variance of balanced incomplete blocks at 5, 10, 30, 60, and 90 min. The effects of ketamine on maximal hormonal responses to an insulin challenge were analyzed via a paired Student t test. Maximal hormonal response to an insulin-induced hypoglycemia was verified by use of a sign test.

Results. The effects of different doses of

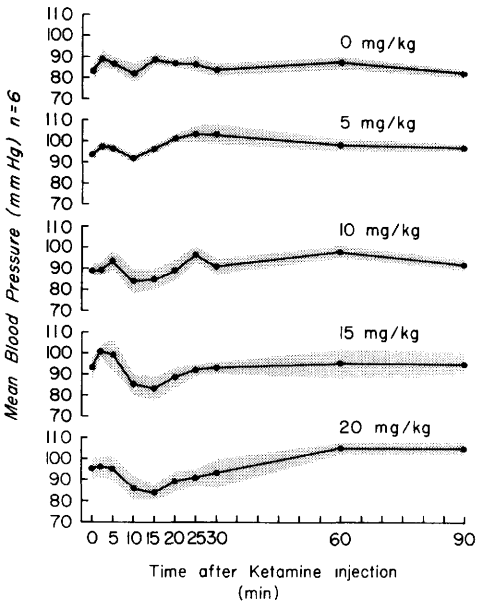

FIG. 1. Effects of various doses of ketamine–HCl on mean blood pressure. Shaded areas represent standard errors of the mean. Changes in mean blood pressure were not significant ($P < 0.20$).

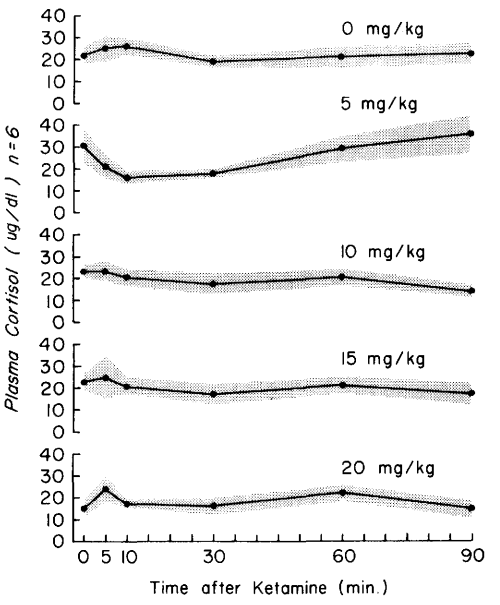

FIG. 2. Effects of various doses of ketamine–HCl on basal plasma cortisol levels. Shaded areas represent standard errors of the mean.

ketamine on mean arterial blood pressure are seen in Fig. 1. We found no evidence that blood pressure changed significantly with any of the ketamine doses ($P > 0.20$), although a slight decrease (<10 mm Hg) was observed at all doses. Likewise, there was no evidence that plasma cortisol, insulin, or blood glucose concentrations changed significantly with the ketamine doses (Figs. 2, 3, and 4) or that ketamine had any effect on heart rate (data not shown). In addition, none of these parameters was significantly altered when the data were expressed as a percentage change from the baseline value of each animal. Thus, ketamine did not affect basal plasma concentrations of insulin, cortisol, or glucose, nor did it have a significant effect on mean arterial blood pressure.

Endocrine responses to insulin-induced hypoglycemia are shown in Fig. 5 and in Table I. No significant differences in endocrine response ($P > 0.20$) existed between ketamine-treated and control animals. In all instances blood glucose levels fell within the first 15–30 min. A maximal decrease in blood glucose of 42 ± 4 mg/dl (mean ± SE) from a control value of 65 ± 4 mg/dl was obtained within the first half-hour. At this time, both plasma GH and ACTH were elevated, although a maximal ACTH response of 195 ± 15 pg/ml^{-1} was obtained at 60 min (Fig. 5, Table I). Plasma cortisol concentrations, although variable, were all elevated at 90 min postinsulin injection (Table I). All maximal hormone responses to an insulin challenge were significant ($P < 0.01$). Thus, ketamine anesthesia did not change the magnitude of hormonal responses to an insulin-induced hypoglycemia nor did it affect the time course in which these hormonal responses occurred.

Discussion. Ketamine–HCl, a phencyclidine derivative, produces a state of dissociative anesthesia by interrupting certain association pathways in the brain before producing somesthetic sensory blockade. This anesthetic is widely used in veterinary medicine for many routine surgical procedures in various species.

Various studies indicate that ketamine has significant cardiovascular and/or endocrine effects in primate and nonprimate species. Other studies indicate insignificant endocrine or cardiovascular effects of

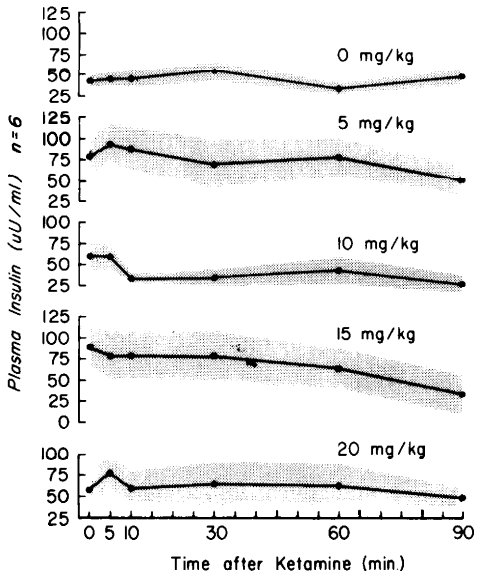

FIG. 3. Effects of various doses of ketamine–HCl on basal plasma insulin levels. Shaded areas represent standard errors of the mean.

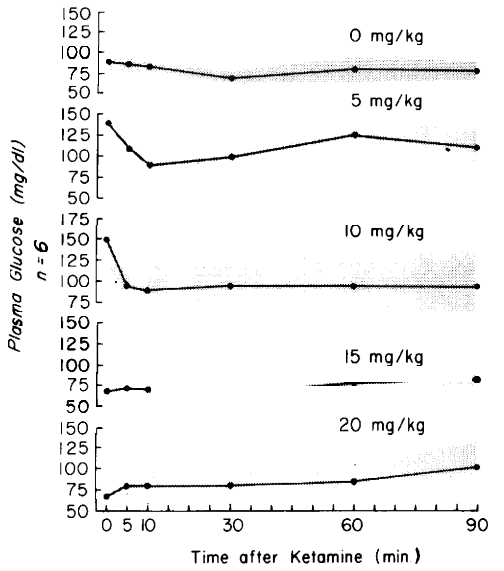

FIG. 4. Effects of various doses of ketamine–HCl on basal blood glucose levels. Shaded areas represent standard errors of the mean.

ketamine depending on the species and mode of administration. An intravenous injection of ketamine given as a bolus dose to the rat has been shown to cause arterial hypotension, whereas it has little or no effect on arterial blood pressure if given as an intramuscular injection (1). Ketamine in dogs appears to exert a pressor response at small doses and a depressor response at higher doses (2). Ketamine–HCl decreases heart rate and peak left ventricular pressure in rhesus monkeys (3). To our knowledge, none of these studies have adequately distinguished between ketamine effects and effects due to animal handling prior to anesthesia. Most importantly, they have not accounted for the effect of animal handling in control situations when no drug is given, thus complicating the comparison of results obtained in the presence and absence of ketamine anesthesia.

Endocrine parameters may remain constant or may change significantly as a consequence of ketamine administration, depending on the species, the particular hormone in question, and experimental condition. Ketamine has been shown to elicit a significant corticosterone increase in rats (4), but does not seem to affect basal prolactin levels in the rat (5). It has also been shown to increase cortisol levels in humans (6) and to slightly decrease serum free fatty acids and slightly increase blood sugar in children (12). Channing *et al.* (13) have shown that ketamine anesthesia has little effect on reproductive hormones in female rhesus monkeys. However, few studies have focused on the effects of ketamine on other primate endocrine systems. Wickings *et al.* (14) have indicated that ketamine anesthesia raises basal plasma prolactin and cortisol levels and produces higher prolactin plasma levels following a thyrotrophin challenge in adult male rhesus monkeys. Although the unanesthetized monkeys in Wickings' study were accustomed to chair restraint, the stress of repeated venipuncture could have elevated plasma cortisol and prolactin levels. For example, Elvidge *et al.* (15) have shown that handling can have a significant effect on plasma cortisol levels; monkeys which had been trained to be bled unanesthetized had significantly lower plasma cortisol levels than untrained unanesthetized monkeys or untrained anesthetized monkeys. Thus, the act of restraining an untrained animal for venipuncture or for induction of anesthesia

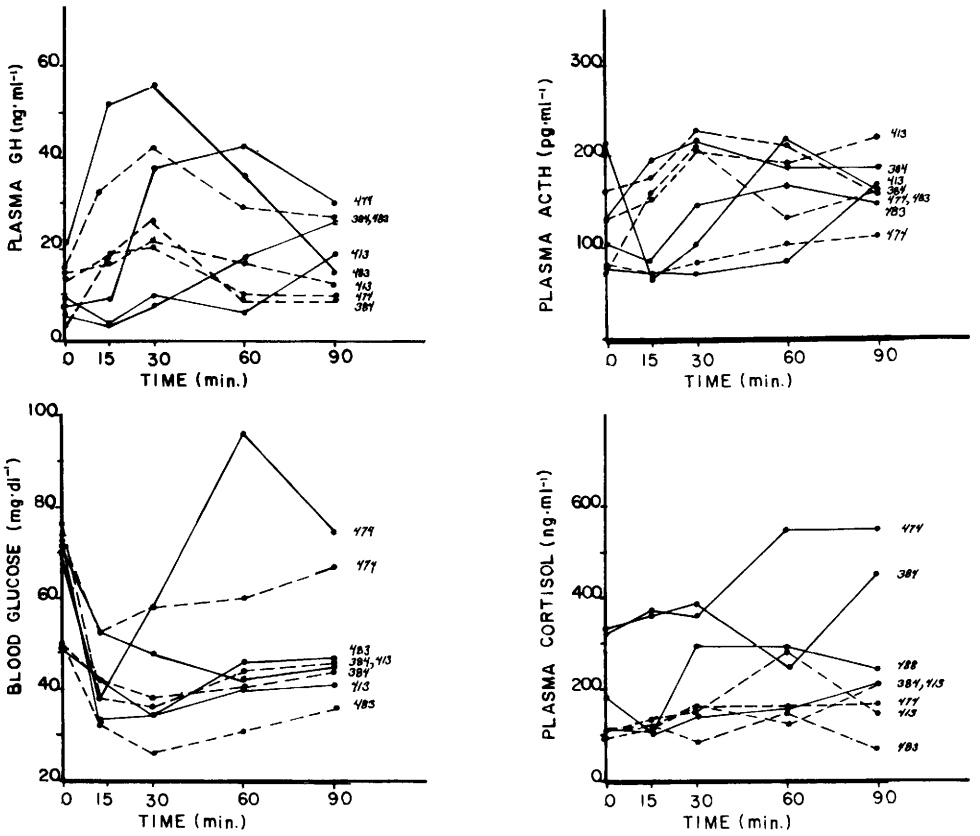

FIG. 5. Endocrine responses to insulin-induced hypoglycemia. Insulin was administered immediately following collection of control blood sample at 0.0 min. Solid lines represent animals receiving ketamine before insulin. Dashed lines represent animals receiving saline before insulin.

could produce significant elevations in certain plasma hormone levels (even if the animal is in a primate chair). Similarly, the method by which blood samples are obtained can influence "basal" plasma hormone levels (15).

Previous studies in nonhuman primates have not exhaustively examined the effects of ketamine on metabolic and endocrine parameters nor have many distinguished between animal handling and ketamine effects. Few studies have used the *M. fascicularis* monkey as the experimental animal. The increasing popularity of *M. fascicularis* in nonhuman primate research prompted us to study the effects of ketamine–HCl on this species under conditions in which the effects of animal handling were tightly controlled. In our study chronic indwelling cannulas allowed a means by which blood pressure could be continuously monitored and blood samples could be obtained without disturbing the animal.

The results of our studies indicate that anesthesia with ketamine does not affect basal plasma levels of insulin, glucose, and cortisol in *M. fascicularis* monkeys. Basal

TABLE I. HORMONAL RESPONSE TO INSULIN-INDUCED HYPOGLYCEMIA

	Control[a]	Maximum response	*N*	*P* value
ACTH (pg·ml⁻¹)	122 ± 16	195 ± 15	8	<0.01
GH (ng·ml⁻¹)	11 ± 2	28 ± 5	8	<0.01
Glucose (mg·dl⁻¹)	65 ± 4	42 ± 4	8	<0.01
Cortisol (ng·ml⁻¹)	171 ± 33	254 ± 54	8	<0.01

[a] Values listed are mean values ± SE of eight pooled observations from ketamine-treated animals and unanesthetized monkeys.

plasma cortisol concentrations at all time periods and at each dosage were similar to those reported by Elvidge *et al.* (15) for their trained unanesthetized monkeys. Untrained monkeys which had received ketamine prior to bleeding had significantly higher plasma cortisol levels than the trained monkeys. Our data show that monkeys receiving ketamine responded no differently from those receiving saline. We conclude that ketamine by itself does not affect basal cortisol levels but that the pituitary–adrenal axis can be significantly activated in animals that are unaccustomed to routine handling. Likewise, ketamine anesthesia does not significantly affect mean arterial blood pressure. The slight decrease in blood pressure at 10 min postinjection may be explained as a response to the intramuscular injection.

Our studies also indicate that ketamine anesthesia does not significantly alter the magnitude of endocrine responses to insulin-induced hypoglycemia, nor does it alter the time course of any of the endocrine responses studied. Therefore, pituitary function, as evidenced by insulin-induced increases in plasma GH and ACTH levels, is unaltered by ketamine. Plasma glucose levels also decreased upon insulin challenge injection, thus showing that metabolic response to insulin are unaltered in ketamine anesthesia. Differences in plasma hormone levels were mostly the result of animal-to-animal variability, regardless of the presence or absence of anesthesia. Our data suggest that ketamine itself does not significantly alter plasma concentrations of insulin, cortisol, or glucose nor does it affect the growth hormone and pituitary–adrenal responses to an insulin challenge.

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