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Plasma Steroid Concentrations in Conscious and Anesthetized Rabbits[1] (39362)

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Many experiments today involve the collection of blood samples from anesthetized animals. Sometimes when these data are compared to those obtained from conscious animals, discrepancies are apparent. It is very possible that some artifacts may be introduced by anesthetics because they are general depressants. For this reason we designed this experiment to examine if there were any significant differences in plasma estrogen (E), androgens (A), and progestin (P) concentrations with time in conscious and anesthetized animals. Furthermore, we compared the steroid responses between animals injected with buffer and LH because the action of gonadotrophins, the release of steroids, and the action of anesthetics all occur at the membrane level (1, 2). Animals were anesthetized with either halothane or sodium pentobarbital (Nembutal). These anesthetics were chosen because they are commonly used anesthetics and have different modes of action (3).

Materials and methods. Adult female rabbits, weighing 3.5 to 5.0 kg, which had free access to water and feed, were used. At least 21 days before use, rabbits were isolated in individual cages. To facilitate the sampling of blood, silastic cannulas were introduced into the right jugular under nembutal anesthesia and externalized dorsally between the ears. Six to nine animals were used for each treatment group. Rabbits were randomly assigned to one of three groups, conscious, nembutal- or halothane-anesthetized, and bled two days after the insertion of cannulas. Anesthesia was induced by either injecting (iv) approximately 2.5 cc of nembutal (Holmes Laboratories) or by inhalation of halothane (Ayerst) in a close circuit (Foregger). Initially for several minutes halothane and oxygen were presented at a rate of 200 cc/*M*% and L/M but were then reduced to 5 cc/*M*% and 0.250 L/M respectively. The level of anesthesia was identical to that used for general surgery. Blood was removed at a constant rate of 0.5 ml per minute by means of an infusion pump. Animals were bled for 15-min intervals before and at 10 and 45 min after the injection of buffer or LH (10 μg/kg) (NIH-S18). After the blood was centrifuged, plasma was removed, frozen, and later assayed for E, A, and P. Statistical analysis was done by analysis of variance.

Results. In Fig. 1 are shown the E, A, and P plasma concentrations before and after the injection of buffer into rabbits that were either conscious, or anesthetized with nembutal or halothane. The E concentrations showed no time or treatment effect. On the other hand, there was some treatment effect observed for A. While there were no significant differences between conscious and halothane-anesthetized animals, plasma levels were significantly different ($P < 0.01$) between the nembutal and halothane-anesthetized animals. However, the greatest treatment effect was noted in the plasma P concentrations. Here the P levels in halothane-anesthetized animals were significantly ($P < 0.01$) elevated over those for the conscious and nembutal-anesthetized rabbits.

In the second experiment we were curious to see if the conscious or anesthetized condition affected the steroid levels following an injection of LH (10 μg/kg). As shown in Fig. 2, no differences were observed in E concentration among the various treatments either before or after the injection of LH. In contrast, significant increases ($P < 0.01$) in A and P occurred after the injection of LH. Though there was a further increase in A and P with time (45–60-min sampling period), the increase was not significantly different from the previous sampling period (10–25-min). A and P concentrations in the conscious and anesthetized animals after LH were not different. In other words, the

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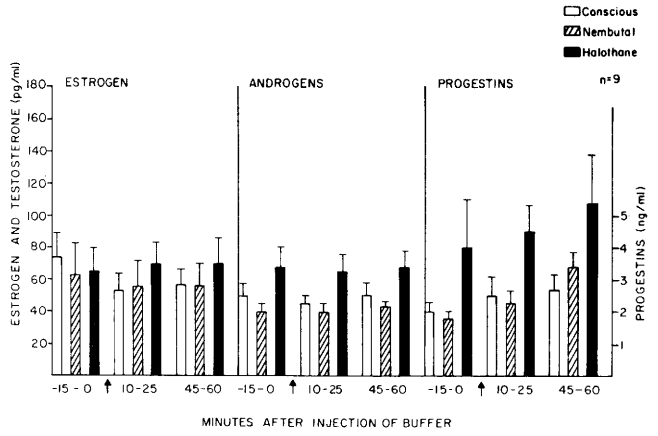

FIG. 1. Peripheral steroid concentrations in conscious and nembutal- and halothane-anesthetized animals before and after the injection of buffer. *n* Indicates the number of animals used in each group. Vertical bars indicate standard errors. The arrow indicates the time of injection of buffer.

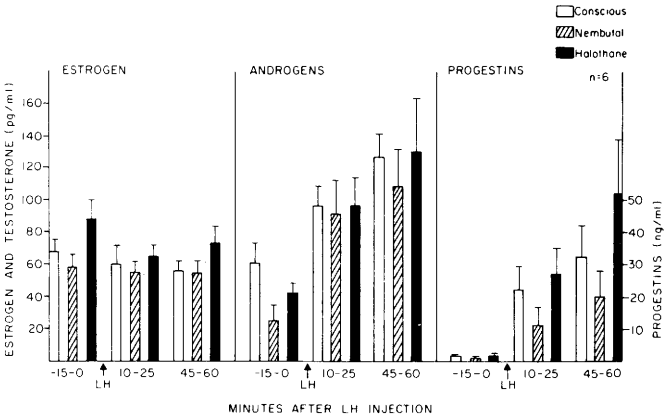

FIG. 2. Peripheral steroid concentrations in conscious and nembutal- and halothane-anesthetized animals before and after the injection of LH. *n* Indicates the number of animals used in each group. Vertical bars indicate standard errors. The arrow indicates the time of injection of LH.

condition of the animal did not affect the steroid response to gonadotrophin stimulation.

Discussion. In these experiments we measured plasma E, A, and P concentration in rabbits that were either conscious or anesthetized with nembutal or halothane. We also compared steroid responses in intact rabbits injected with either buffer or LH. Of the three steroids measured plasma E levels were not altered either by the state of the animal (conscious or anesthetized) or following gonadotrophin stimulation. Since changes in E levels following a systemic injection of LH as measured in the ovarian venous blood increase only twofold (unpublished data), it is not surprising that there is no change in peripheral plasma E concentrations following LH. Similarly, any changes caused by anesthesia may be so minute as not to be observed in the peripheral plasma. On the other hand, A levels were slightly, but not significantly, elevated under halothane anesthesia in Experiment 1. For some unexplainable reason this response was not seen in the first group of samples taken in the second experiment (see Fig. 2) before the injection of LH. This variation in A levels may reflect the variation among the animals or may result from the pooling of the data. The slightly elevated androgen levels observed in halo-

thane-anesthetized animals in Experiment 1 do agree with the results, but not the interpretation, of Free and Tillson (4). They measured secretion rates of testosterone in conscious and halothane-anesthetized rats and observed slightly lower testosterone levels in the conscious rats which they attributed to stress in the conscious rats. On the other hand, while we did not observe any highly significant changes in A levels, Oyama (5) and Oyama *et al.* (6), reported that testosterone concentrations in human plasma decreased 12% from control values during 45 min of halothane anesthesia and continued to fall to 80% of the preinduction levels. These low values continued to fall and persisted for 1 week postoperatively. It is difficult, if not impossible, to draw any comparison among these experiments because of differences in species and anesthetics used, sampling procedures, levels of surgical stress, and duration of the anesthesia.

P concentrations in the halothane-anesthetized rabbits were significantly elevated over those measured in conscious and nembutal-anesthetized rabbits in Experiment 1. In Experiment 2, in which rabbits were injected with LH, this treatment effect, though most likely present, was overridden by the tremendous increase in P levels following gonadotrophin stimulation. These significant differences in P levels in Experiment 1 between the nembutal and halothane anesthetized rabbits may reflect the different physiological response to the two anesthetics. While nembutal is a barbiturate and general depressant, halothane, a voltatile anesthetic, induces delirium unless sedation is first induced by a barbiturate.

In conclusion, we found that P levels were elevated in animals anesthetized with halothane. This highly significant increase in P, but not in E and A, may be the result of stress. We are now investigating whether the ovary or the adrenal is the principal source of the elevated P levels in halothane-anesthetized animals. However, one must be cautious in extrapolating these results to other species because of species differences and the lack of sufficient data regarding hormonal responses following induction of anesthesia.

Summary. The ovarian steroids, estrogen, androgen, and progestin, were measured in the peripheral plasma of adult female rabbits that were either conscious or anesthetized with sodium pentobarbital (nembutal) or halothane. Concentrations of estrogen, androgens, and progestin were determined before and at 10 and 45 min after systemic injection of either buffer or LH. In the controls androgen levels were significantly different between animals anesthetized with nembutal and halothane. However, the greatest treatment effect was noted in plasma progestin concentrations which were significantly elevated in halothane-anesthetized animals in comparison to the conscious and nembutal-anesthetized animals. Following LH, the androgen and progestin levels were significantly elevated over basal levels. Most likely the treatment effect observed in the controls was still present but was overridden by the increased release of steroids following gonadotrophin stimulation. This study suggests that halothane, in contrast to nembutal, does significantly elevate peripheral progestin and androgen levels.

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