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Effects of Dexmedetomidine and Ketamine–Dexmedetomidine with and without Buprenorphine on Corticoadrenal Function in Rabbits

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Anesthetics may influence adrenal function and consequently alter serum glucocorticoid concentrations, leading to erroneous interpretations of results from anesthetized rabbits. However, decreases in glucocorticoid concentrations may be advantageous in protocols designed to minimize the stress response to surgery. This study characterized the variations in adrenocortical function based on changes in corticosterone and cortisol levels after various doses and combinations of dexmedetomidine, ketamine, and buprenorphine. Each rabbit received all treatments with a minimal interexperiment interval of 10 d. Rabbits were allocated to 7 groups (n = 10 per group) and received either 1 mL saline solution; dexmedetomidine at 0.05, 0.15, or 0.25 mg/kg; ketamine (35 mg/kg) and dexmedetomidine (0.25 mg/kg) without or with buprenorphine (0.03 mg/kg); or ketamine (35 mg/kg) and buprenorphine (0.03 mg/kg). Blood was sampled before drug administration and at 10, 30, 60, and 120 min and 24 h afterward. Serum glucocorticoid levels fell in all treatment groups except the one receiving ketamine–dexmedetomidine; in that group, serum glucocorticoids increased. Rabbits that received ketamine–dexmedetomidine–buprenorphine had the lowest serum glucocorticoid levels overall. In conclusion, dexmedetomidine reduces glucocorticoid secretion in rabbits but, when combined with ketamine, increases corticosterone and cortisol levels as well as heart and respiratory rates. The addition of buprenorphine to the ketamine–dexmedetomidine mixture reduces serum glucocorticoid levels. The influence of anesthetic drugs should be considered when designing a protocol to minimize the glucocorticoid response to surgery or when measuring glucocorticoid levels in rabbits.

Abbreviations: ACTH, adrenocorticotrophic hormone; CRH, corticotrophin-releasing hormone; HPA, hypothalamic–pituitary–adrenal.

Minimizing the pain, distress, and suffering of laboratory animals is a legal and ethical imperative. Procedures causing actual or potential suffering to animals should be done under appropriate analgesia, sedation, or anesthesia. Research protocols and clinical requirements support the selection of the most appropriate analgesics and anesthetics.[22] However, commonly used anesthetics can affect numerous physiologic parameters in laboratory animals. Rabbits are easily stressed by incorrect preoperative handling and the induction of anesthesia.[12] However, despite the involvement of the adrenal gland in short- and long-term adaptation of organisms to stress-inducing agents, little is known about the effect of anesthesia on corticoadrenal function in rabbits.

The stress response to surgery comprises a number of hormonal changes initiated by neuronal activation of the hypothalamic–pituitary–adrenal (HPA) axis.[10] Faciliatory and inhibitory pathways—involving GABAergic, cholinergic, adrenergic, dopaminergic, and serotoninergic systems—are all involved in the hypothalamic regulation of corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and therefore cortisol secretion.[13] Anesthetics may influence these systems and consequently alter serum glucocorticoid concentrations, leading to the erroneous interpretation of results when measuring glucocorticoid levels in anesthetized rabbits. However, anesthesia-associated decreases in cortisol and corticosterone levels might be advantageous when designing a protocol to minimize the stress response to surgery.

We performed the present study to characterize features of the response to surgery in rabbits, specifically measuring changes in heart and respiratory rates and in serum corticosterone and cortisol concentrations after various doses of dexmedetomidine and ketamine–dexmedetomidine with and without buprenorphine. Dexmedetomidine ([+]4-[1-{2,3-dimethylphenyl}-ethyl]-1H-imidazole) is a potent and highly selective α2 adrenoreceptor agonist with sympatholytic, sedative, and analgesic properties that has been described as a useful and safe adjunct in many clinical applications[2] and comprises only the active molecule of its predecessor, medetomidine.[26] Ketamine is classified as an N-methyl-D-aspartate receptor antagonist that produces a dissociative anesthetic state and that indirectly stimulates cardiovascular function.[4] Buprenorphine is a semisynthetic alkaloid of opium with analgesic properties; it may be beneficial during the postoperative period by reducing the depressant effects of surgery on food and water consumption, as related to the presence of postoperative pain.[9,27]

Our hypothesis was that various anesthetic mixtures containing dexmedetomidine would alter serum glucocorticoid levels in rabbits. Such effects should be considered during the evaluation of experimental results from rabbits treated with these

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agents, as they could confound the interpretation of results and lead to erroneous conclusions. However, an anesthesia-associated decrease in adrenal function could be exploited to stabilize the stress response during surgery or the postoperative period, thus contributing to animal wellbeing.

Materials and Methods

Animals. The study involved female New Zealand white rabbits (*Oryctolagus cuniculus*; *n* = 10; age, 7 mo; weight, 2.5 to 3.5 kg; Granja San Bernardo, Navarra, Spain). Treatment order was randomized, and each rabbit received all treatments with at least 10 d between experiments. The rabbits were housed in individual wire-rod–floored, stainless-steel cages each measuring 48 × 61 × 46 cm, with a collection pan beneath each cage and in a room with controlled environmental conditions (20 to 22 °C; 50% to 55% relative humidity; 10 to 15 air changes per hour; and a 12:12-h light:dark cycle). In the procedure room, the temperature was 20 to 22 °C. The rabbits were quarantined 15 d prior to use to permit adaptation to environmental conditions, food, and water and to allow daily evaluation of health status. All of the rabbits were clinically healthy prior to the study and were free of recognized pathogens (*Pasteurella multocida*, *Bordetella bronchoseptica*, *Trychophyton microsporum*, *Escherichia coli*, coccidia, ectoparasites, and endoparasites). The rabbits were fed a standard rabbit diet (150 g daily; Lab Rabbit Chow, Purina, Barcelona, Spain), and fresh water was provided free choice. To minimize stress reactions, the rabbits were handled (placed into the restraining cage and blood samples of 1 mL collected) daily for 1 wk before the beginning of experiments. All experimental manipulations were performed between 09:15 and 12:45. The operator was blinded to the treatment group assignment of each rabbit at all times. The experimental protocols were approved by the IACUC of the Veterinary Medicine School at Complutense University of Madrid (Spain). All procedures were completed in accordance with the *Guide for the Care and Use of Laboratory Animals*[22] and conformed with the relevant European Union Directive.

Experimental design. On the day of each experiment, each rabbit was weighed 1 h before physiologic variables were measured. Conscious rabbits were placed into restraining cages for catheter insertion and during the intramuscular injection for induction of anesthesia. A 24-gauge intravenous catheter (Therumo, Leuven, Belgium) was placed in the marginal ear vein under local anesthesia (EMLA cream, AstraZeneca, Madrid, Spain), which was applied to the ear 45 to 60 min before blood collection.

The groups (*n* = 10 in each group of treatment, across all time points) and treatments were: group C (controls), 1 mL normal saline solution; group D05, dexmedetomidine (Dexdomitor, Orion Pharma, Pfizer, Espoo, Finland) at 0.05 mg/kg IM; group D15, dexmedetomidine at 0.15 mg/kg IM; group D25, dexmedetomidine at 0.25 mg/kg IM; group KD, ketamine (35 mg/kg IM; Imalgene 1000, Merial Laboratories, Barcelona, Spain) and dexmedetomidine (0.25 mg/kg IM); and group KDB, ketamine (35 mg/kg IM), dexmedetomidine (0.25 mg/kg IM), and buprenorphine (0.03 mg/kg IM; Buprex, Schering-Plough, Madrid, Spain). The effects of ketamine (35 mg/kg IM) and buprenorphine (0.03 mg/kg SC) without dexmedetomidine (group KB) was studied to clarify the effect of dexmedetomidine compared with buprenorphine in our research. We chose not to include a dexmedetomidine–buprenorphine group in the experimental design because we surmised that the use of this particular combination would not result in a noteworthy, novel outcome. For groups KD and KDB, dexmedetomidine and ketamine were mixed in the same syringe. The doses were based on results from preliminary studies and a review of previous anesthetic studies using laboratory rabbits.[19,30] All drugs were injected into the quadriceps femoris, except for buprenorphine, which was injected subcutaneously. The maximal volume administered intramuscularly to the rabbits was 0.85 mL/kg (KD and KDB groups). Buprenorphine was administered 30 min before ketamine–dexmedetomidine (group KDB) or ketamine (group KB) injection.

The depth of anesthesia was monitored by using the pedal withdrawal, ear pinch, and righting reflexes.[18] According to previous studies, surgical anesthesia was judged to be present when there was an absence of response to ear-pinching and pedal withdrawal.[19,30] When assessing these reflexes, any movement was considered to be a positive response. Samples (approximately 2 mL) of blood were collected from the intravenous catheter at 6 time points: just before drug administration and at 10, 30, 60, and 120 min and 24 h after injection. Blood was replaced with 3 times the volume of lactated Ringer solution; an average volume of 2 mL/kg, administered through an intravenous catheter (approximately 6 mL per bolus during 1 min), was provided just after the sample collection time points. Conscious rabbits were placed into restraining cages for administration of lactated Ringer solution. Blood samples were maintained in blood collection tubes with no additives for 2 h at 20 to 22 °C and then centrifuged (Minifuge RF, Heraeus, Hannover, Germany) at 1200 × *g* and 4 °C for 20 min. Serum was separated and stored frozen at –30 °C until assayed.

Variables measured. Heart rate was determined from a lead II electrocardiogram recording (Bexgraph, Bexen-Osatu, Vizcaya, Spain). Self-adhesive ECG electrodes (Lessa, Barcelona, Spain) were placed on the skin on the medial aspect of the upper forelegs and left hindleg of the rabbits. Rabbits were spontaneously breathing and supplemented with oxygen (2 L/min) during anesthesia from a face mask. Respiratory rate was assessed visually by counting respirations over a 30 s period. Body temperature was monitored by using a rectal probe (model 0331, Panlab, Barcelona, Spain) and maintained at 36.5 to 38.8 °C throughout anesthesia and recovery by placing the animals on an electric heating pad (B Braun Vet Care, Barcelona, Spain) set at 40 °C and covered with a towel to reduce the risk of skin burns.

Serum corticosterone and cortisol levels were measured in each blood sample by using a competitive enzyme immunoassay previously validated for this species.[21] Lower detection limits were 0.01ng/mL for cortisol and 0.15 ng/mL for corticosterone.

Statistical analysis. Statistical analysis was performed by using commercially available computer software (version 19 for Windows, SPSS Statistics, IBM, Chicago, IL). Two-way ANOVA (treatment × time) with repeated measures on both factors was done to determine the effects of treatment and time on the values of the parameters we measured and the interaction between them. When a significant interaction was found between factors, the effect of each of them on the correspondent parameter was assessed by using a one-way ANOVA with repeated-measures analysis independently for each of the levels of the other factor. A Bonferroni posthoc test was performed. Differences were considered significant when the *P* value was less than 0.05. The parametric results are expressed as mean ± 1 SD.

Results

Reflexes. After dexmedetomidine injection (groups D05, D15, and D25), rabbits were only sedated and responded

to pedal withdrawal and ear pinch reflexes. Induction and recovery of anesthesia, denoted by the loss and recovery of the pedal withdrawal reflex respectively, were smooth and trouble-free in groups KD, KDB, and KB, in which all rabbits lost the 3 monitored reflexes within 10 min of injection. The times required for the return of the righting, pedal withdrawal, and ear pinch reflexes in group KDB were 129.4 ± 22.6, 72.6 ± 26.6, and 74.2 ± 25.3 min, respectively in group KD were 73.2 ± 19.6, 50.1 ± 19.8, and 51.7 ± 21.7 min, respectively; and in group KB were 81.1 ± 21.6, 57.1 ± 20.1, and 56.1 ± 16.8 min, respectively. The time required for the return of the righting reflex was greater in group KDB than in groups KD and KB ($P < 0.05$ in both cases).

Cortisol and corticosterone levels. Serum glucocorticoid levels were equivalent among groups at the 0-min time point (Figure 1). Serum cortisol levels at 10, 30, 60, and 120 min were lower in group KDB than in groups C ($P < 0.05$ in all cases) and KD ($P < 0.001$ at 10 and 120 min; $P < 0.05$ at 30 and 60 min) and at 10 and 30 min than in groups D05, D15, D25, and KB ($P < 0.05$ in all cases). However, serum cortisol was increased in group KD at 10 and 120 min when compared with all other groups ($P < 0.001$ in all cases). Moreover, serum cortisol levels at 120 min were greater in group C than in group D05 ($P = 0.04$), D15 ($P = 0.022$), and KDB ($P = 0.020$; Figure 1).

Serum corticosterone levels were greater in group KD than in all other groups at 10, 30 and 120 min ($P < 0.001$ in all cases except with group D25 at 120 min, $P = 0.002$; Figure 1). In addition, serum corticosterone concentrations fell at 60 min in groups D05 and D25 compared with groups C ($P = 0.039$ and $P = 0.046$, respectively) and D15 ($P = 0.008$, $P = 0.026$, respectively; Figure 1) and was decreased at 60 min in group KDB compared with groups C ($P = 0.011$), D15 ($P < 0.001$), KD ($P = 0.031$), and KB ($P = 0.038$). Serum glucocorticoid levels were similar among groups at 24 h after administration of anesthesia.

Heart and respiratory rates. There were no differences in heart rates among groups at the 0-min time point (Figure 2). However, at 10 min after injection, heart rate was decreased ($P < 0.01$ in all cases) in all treatment groups when compared with the baseline (time 0) rate or that of control group (group C) at the 10-min time point. This decrease was maintained in all treatment groups until 120 min after drug administration, with exception of group KD at 120 min (Figure 2). Heart rate was increased in group KD over the values for group D15 ($P = 0.001$ at 30 min; $P = 0.002$ at 60 and 120 min) and D25 ($P < 0.001$ at 10, 30, and 60 min; $P = 0.006$ at 120 min) and for group KDB at 120 min ($P < 0.001$). In group D25, heart rate between 10 and 60 min was decreased when compared with that of groups C, D05, and KD ($P < 0.001$ in all cases), KDB ($P = 0.028$ at 10 min; $P = 0.002$ at 30 min), and KB ($P < 0.001$ at 10 and 30 min; $P = 0.01$ at 60 min).

There were no differences in respiratory rate among groups at the 0-min time point (Figure 2). Respiratory rate decreased ($P < 0.01$ in all cases) between 10 and 120 min in all treatment groups when compared with the baseline (time 0) rate or that of group C at the same time point, exception of group KD at 120 min ($P > 0.05$). In addition, respiratory rate at 60 min was lower in group KDB when compared with groups D05 ($P = 0.012$), D25 ($P = 0.014$), and KD ($P < 0.001$). At 120 min, respiratory rate was increased in group D05 over values for group KDB ($P = 0.002$) and in group KD when compared with groups D15 ($P = 0.002$), D25 ($P = 0.024$), and KDB ($P < 0.001$).

Rectal temperature. During the maintenance of anesthesia in groups KD, KDB, and KB, mean rectal temperature remained similar ($P > 0.05$) among all groups.

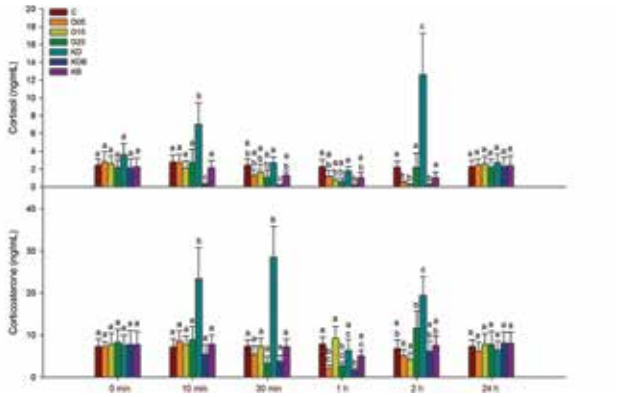

Figure 1. Effects of various anesthetic–sedative protocols on serum cortisol (ng/mL) and corticosterone (ng/mL) at various time points after intramuscular injection of saline solution (group C); dexmedetomidine (0.05 mg/kg, group D05; 0.15 mg/kg, group D15; or 0.25 mg/kg, group D25); ketamine (35 mg/kg) + dexmedetomidine (0.25 mg/kg, group KD); ketamine (35 mg/kg) + dexmedetomidine (0.25 mg/kg) + buprenorphine (0.03 mg/kg SC, group KDB); and ketamine (35 mg/kg) + buprenorphine (0.03 mg/kg SC, group KB) in rabbits ($n = 10$ group). All values are expressed as mean ± 1 SD. Different letters denote significant differences ($P < 0.05$) between groups at the same time point.

Discussion

Our study shows the changes in selected physiologic variables in rabbits, including serum glucocorticoids, after different doses of dexmedetomidine, ketamine–buprenorphine, and ketamine–dexmedetomidine combinations with and without buprenorphine.

Rabbits that received different doses of dexmedetomidine showed no loss of measured reflexes, but reflexes were absent in groups KD, KDB, and KB. These reflexes were suppressed longer in group KDB as compared with groups KD and KB. Premedication with buprenorphine significantly prolonged the time of anesthesia induced by ketamine–medetomidine in rabbits.[30]

Serum glucocorticoid concentrations are regulated by both the peripheral system, in the adrenal cortex, and the CNS, through the release of CRH and ACTH. In response to stress, CRH is released into hypophyseal portal vessels that access the anterior pituitary gland. Binding of CRH to its receptor induces the release of ACTH into the systemic circulation. The principal target for circulating ACTH is the adrenal cortex, where it stimulates glucocorticoid synthesis and secretion. Serum glucocorticoid levels in rabbits fell after various doses of dexmedetomidine, the active D-isomer of the selective α2 agonist medetomidine. Whether this effect is specific for α_2-adrenoceptors and imidazoline receptors or extends to other receptors is unclear. Some studies suggest that imidazoline receptors may be involved in the inhibition of cortisol secretion from the adrenal cortex[24] and in steroidogenesis perioperatively.[28] Dexmedetomidine decreased serum glucocorticoid concentrations[6] and attenuated the corticoadrenal response to several stressors, such as surgery and intubation, in humans.[29,36] In dogs, basal cortisol levels decreased and the cortisol response to ACTH was blunted 3 h after dexmedetomidine administration.[28] However, our results in rabbits show that similar effects on corticosterone and cortisol do not appear to be dose-related. Other authors have not noted changes in glucocorticoid levels after dexmedetomidine administration in dogs,[33] and dexmedetomidine infusion did not inhibit adrenal steroidogenesis when the drug was used for short-term sedation after surgery in humans.[38]

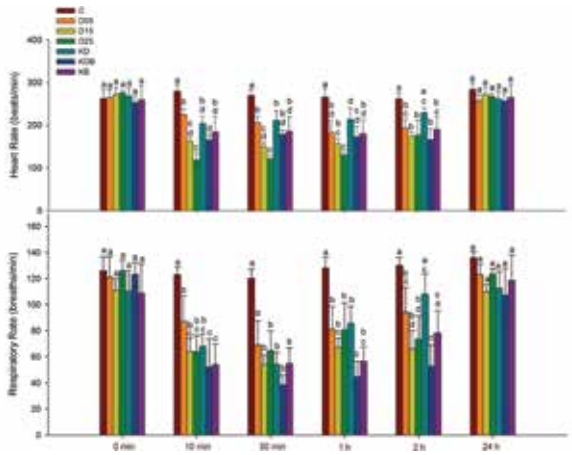

Figure 2. Effects of various anesthetic–sedative protocols on heart rate (beats/min) and respiratory rate (breaths per minute) at various time points after intramuscular injection of saline solution (group C); dexmedetomidine (0.05 mg/kg, group D05; 0.15 mg/kg, group D15; or 0.25 mg/kg, group D25); ketamine (35 mg/kg) + dexmedetomidine (0.25 mg/kg, group KD); ketamine (35 mg/kg) + dexmedetomidine (0.25 mg/kg) + buprenorphine (0.03 mg/kg SC, group KDB); and ketamine (35 mg/kg) + buprenorphine (0.03 mg/kg SC, group KB) in rabbits (n = 10 group). All values are expressed as mean ± 1 SD. Different letters denote significant differences ($P < 0.05$) among groups at the same time point.

The combination of dexmedetomidine and ketamine was associated with increases in both cortisol and corticosterone in rabbits. Thus, dexmedetomidine may have partially inhibited the ketamine-induced increases in serum glucocorticoids. In fact, our results indicate that dexmedetomidine alone, at different doses, decreased glucocorticoid levels. N-methyl-D-aspartate receptors are involved in the physiologic pulsatile regulation of hormone release from the HPA axis.[5] Ketamine, an antagonist of N-methyl-D-aspartate, increases serum cortisol levels through a sympathomimetic effect in humans.[20] Ketamine also increased serum glucocorticoids in rats and dogs.[3,25] Moreover, when used as the sole anesthetic, ketamine strongly activates the sympathoadrenergic system, leading to increases in plasma levels of catecholamines.[1] In previous studies, we demonstrated an increase in serum corticosterone concentrations after ketamine–medetomidine anesthesia in rabbits.[15]

However, the intramuscular injection of ketamine can be painful and causes muscle necrosis in small mammals.[7] Subcutaneous injection of buprenorphine 30 min prior to injecting ketamine may have decreased this painful stimulus in KDB rabbits, leading to the dampened increase in both glucocorticoids in group KDB compared with KD. Therefore, ketamine injection may cause pain and thus trigger glucocorticoid secretion in conscious rabbits. Buprenorphine likely decreases the pain due to intramuscular injection with ketamine. Additional studies should be performed to determine whether the increase in glucocorticoid levels after ketamine–dexmedetomidine injection mainly reflects a response to the pain of ketamine injection or ketamine-induced stimulation of adrenocortical function.

Our results revealed that the addition of buprenorphine to ketamine or the ketamine–dexmedetomidine mixture decreased the serum concentrations of both cortisol and corticosterone concentrations and therefore likely inhibited corticoadrenal function. In rats, buprenorphine did not activate either the HPA axis, which would have been apparent as glucocorticoid release, or the sympathetic nervous system, which would have led to catecholamine production.[17] The role of multiple opioid receptors in the opioid-induced changes in anterior pituitary hormone release has been demonstrated in rats.[32] In addition, buprenorphine treatment decreased plasma corticosterone levels in mice and rats[16,23] and inhibited the HPA axis in rats.[14] Moreover, buprenorphine attenuated the corticoadrenal response to several stressors, such as surgery, in rodents.[16] However, other authors did not find buprenorphine-associated differences in the glucocorticoid levels of mice and rats.[32,37]

Serum glucocorticoid levels were lower in group KDB rabbits than in those in group KB. This finding suggests that dexmedetomidine has a suppressive action on both cortisol and corticosterone in group KDB. However, serum glucocorticoid concentrations decreased after KB administration compared KD. Therefore, the effect of dexmedetomidine was probably minor compared with that of buprenorphine. One limitation of our study is the lack of a trial investigating the influence of the dose of ketamine and buprenorphine, to determine the dose-effect of these drugs on corticoadrenal function.

In our study, both heart and respiratory rates decreased in dexmedetomidine-treated groups of rabbits. The values we obtained were in agreement with those reported by other authors for rabbits given dexmedetomidine.[31] Dexmedetomidine causes bradycardia and bradypnea, probably due to decreased sympathetic tone and secondary to CNS depression.[35] However, when dexmedetomidine was administered together with ketamine, the decrease in heart and respiratory rates was less pronounced, probably reflecting ketamine-associated inhibitory effects on the parasympathetic system and stimulatory sympathomimetic effects.[4,11] The addition of buprenorphine to ketamine or the dexmedetomidine–ketamine mixture might counteract this stimulatory effect through the depression of sympathetic nerve activity, but this buprenorphine-induced depressive effect has not been established. Some authors reported that buprenorphine (dose, 0.01 to 0.1 mg/kg) can induce bradycardia and bradypnea in rabbits,[8,30] whereas others supported that buprenorphine (0.06 mg/kg) does not change heart rate in rabbits.[34] Mechanical ventilation of the animals is necessary in cases of insufficient gas exchange to minimize a hypoxic stress response. Future studies should monitor blood pressure and pulse oximetry to establish the relationship between blood levels of stress hormones and clinical indicators of stress.

These results led us to several conclusions. First, the effects of anesthetic drugs on glucocorticoid responses should be considered when designing a protocol. Second, the use of α2 adrenergic agonists, such as dexmedetomidine, and especially opioid analgesics, such as buprenorphine, to inhibit the increased adrenocortical function caused by other anesthetic drugs, such as ketamine. Third, anesthesia-induced changes in glucocorticoid levels, if not recognized, could lead to erroneous interpretation of results in rabbits treated with these anesthetic mixtures. Future studies should explore the influence of various anesthetic combinations on the outcomes of various surgical procedures in rabbits.

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