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Corticoadrenal and Cardiorespiratory Responses to Administration of Propofol Combined with Dexmedetomidine or Ketamine in Rabbits

Alfredo González-Gil,[1,*] Rosa Ana Picazo,[1] Paul de Bruyn,[2] and Juan Carlos Illera[1]

Anesthetic protocols may influence adrenal function. Effective methods for modulating stress are desirable to minimize secondary effects during the perioperative period. The aim of this study was to evaluate the effects of the administration of propofol with dexmedetomidine or ketamine on corticoadrenal function and heart and respiratory rates. A random treatment-order design was used: each rabbit received all treatments, with at least 14 d between experiments. Rabbits were assigned to 3 treatment groups (10 per group): group 1, 1 mL normal saline solution intravenously; group 2, propofol (3 mg/kg IV) and dexmedetomidine (0.35 mg/kg IM); and group 3, propofol (3 mg/kg IV) and ketamine (1 mg/kg IV). Dexmedetomidine was injected 15 min prior to propofol administration. Blood samples were obtained before drug administration and at 5, 10, 30, and 60 min and 24 h after injection. Serum cortisol and corticosterone levels were measured by competitive enzyme immunoassay. Serum glucocorticoid concentrations did not change in group 2. However, rabbits in group 3 showed an increase in serum cortisol (at 5-60 min) and corticosterone (at 5-120 min) when compared with all other groups at the corresponding time points. This increase probably reflected both propofol- and ketamine-associated stimulatory effects corticoadrenal function. Respiratory rate decreased in groups 2 and 3 animals, and heart rate decreased in group 2, probably due to sympathetic inhibition by propofol and dexmedetomidine. In conclusion, propofol–ketamine provides suitable cardiorespiratory stability in rabbits but enhances glucocorticoid secretion more than dexmedetomidine–propofol anesthesia. Glucocorticoid levels in anesthetized rabbits should be considered during protocol design to minimize the stress response to surgery and to avoid erroneous data interpretation.

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 relationship between anesthesia and the hypothalamic–pituitary–adrenal axis is an important consideration when designing an anesthetic protocol to minimize a stress response. Anesthetics may influence this function and, consequently, alter serum glucocorticoid concentrations.[1,15] Importantly, alterations in serum glucocorticoid concentrations may confound the analytic results obtained after anesthesia administration in rabbits. Combining different agents to minimize adverse effects and maximize the beneficial profile of each drug is an important avenue of investigation. Propofol, a sedative–hypnotic drug with anesthetic properties, induces dose-dependent decreases in blood pressure and heart rate and transient hypotension.[22] Dexmedetomidine, a selective α2 adrenoreceptor agonist has been used for sedation–analgesia and to reduce required doses of opioids, propofol, and benzodiazepines.[3] The combination of propofol and dexmedetomidine provides cardiovascular stability with decreased adverse effects, including respiratory depression.[20] Ketamine is a general anesthetic with sedative and analgesic properties and stimulating effects on the cardiovascular and respiratory systems.[23] Alternatively, combining propofol and ketamine results in a more consistent sedation and less hypotension when compared with the use of propofol alone.[2,24]

Recent studies have reported that propofol increases serum glucocorticoids in rabbits.[14] However, the effects on the adrenal function after combining propofol with dexmedetomidine or ketamine have not been well explained in rabbits. To minimize the stress response to surgery and reduce postoperative complications during the perioperative period, anesthesia–associated decreases in glucocorticoid concentrations might be advantageous.

The study aimed to evaluate the effects of propofol combined with dexmedetomidine or ketamine on corticoadrenal function and heart and respiratory rates.

Materials and Methods

Animals. The study involved 10 female New Zealand White rabbits (*Oryctolagus cuniculus*; age, 7 mo; weight, 2.5 to 3.5 kg; Granja San Bernardo, Navarra, Spain). A random treatment-order design was used: each rabbit received all 3 treatments, with at least 14 d between experiments. 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). To accustom them to interaction with humans and to minimize stress, rabbits were handled daily for 7 d prior the onset of the experiment (rabbits were placed into the restraining cage and blood samples of 1 mL collected daily). Animals were housed in individual stainless steel cages (48 × 61 × 46 cm) with wire-grid floors. The rabbits were maintained under conventional conditions (12:12-h light:dark cycle, 20 to

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[1]Department of Animal Physiology, Veterinary Medicine School, Complutense University of Madrid, Madrid, Spain;[2] Department of Research and Statistics, International Commission for Conservation, Madrid, Spain
[*]Corresponding author. Email: aggil@vet.ucm.es

22 °C; 50% to 55% relative humidity; 10 to 15 air changes each hour), were fed a standard rabbit diet (150 g daily; Lab Rabbit Chow, Purina, Barcelona, Spain), and had unrestricted access to fresh water.

Experimental design. Local anesthesia with eutectic mixture of local anesthetics cream (AstraZeneca, Madrid, Spain) was applied 45 to 60 min before catheter insertion. A 24-gauge intravenous catheter was inserted into a marginal ear vein. Six samples (2 mL each) of blood were drawn from the catheter (blood sample at 24 h was collected by using a 23-gauge needle) just before drug administration and at 5, 10, 30, and 60 min and 24 h after injection. All experimental procedures were performed between 0900 and 1300 h. The collected blood was replaced with approximately 2 mL/kg (approximately 6 mL per bolus during 1 min) lactated Ringer solution administered through the catheter, immediately after each sample collection. Ten rabbits were assigned to each group (n = 10/group, each rabbit received all treatments). Each group received one of the following treatments: group 1, control (1 mL normal saline solution intravenously); group 2, dexmedetomidine (0.35 mg/kg IM; Dexdomitor, Pfizer, Espoo, Finland) and propofol (3 mg/kg IV; PropoClear, Fort Dodge, Girona, Spain); and group 3, propofol (3 mg/kg IV, Pfizer) and ketamine (1 mg/kg IV; Imalgene 1000, Merial, Barcelona, Spain). Dexmedetomidine was injected into the quadriceps muscle at 15 min prior to propofol administration. These doses and routes were based on preliminary investigations and a review of other anesthetic studies using rabbits.[8,16] 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. Serum corticosterone and cortisol levels were quantified in each blood sample by using a competitive enzyme immunoassay previously validated for this species.[17] Lower detection limits were 0.15 ng/mL for corticosterone and 0.01ng/mL for cortisol. Surgical anesthesia was considered to be present when ear-pinching and pedal withdrawal reflexes were absent.[15] Righting reflex was also evaluated. Rabbits were breathing spontaneously and were supplemented with 2 L/min of oxygen during anesthesia from a face mask. Heart rate was recorded from a lead II ECG recording, and the respiratory rate was measured by visually counting respirations. Body temperature was monitored by using a rectal probe (model 0331, Panlab, Barcelona, Spain) and maintained at 36.8 to 38.6 °C throughout anesthesia and recovery by placing the rabbits 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. Throughout all experiments, the operator was blinded to treatment group assignment. The experimental protocols were approved by the IACUC of the Veterinary Medicine School at Complutense University of Madrid (Spain). All procedures were in conformity with the relevant European Union Directive 86/609/EEC.

Statistics. Prism 4 (GraphPad Software, San Diego, CA) was used to perform the statistical analysis. Data collected on glucocorticoid levels and heart and respiratory rates were analyzed by using 2-way ANOVA with repeated measures, to determine the effects of treatment and time (and their interaction) on the measured parameters. Bonferroni posthoc testing was performed where appropriate. The differences were considered to be significant when the P value was less than 0.05.

Results

Reflexes. Induction of surgical anesthesia, denoted by loss of the ear-pinch response and pedal withdrawal reflex, was smooth and trouble-free in both the dexmedetomidine–propofol (group 2) and propofol–ketamine (group 3) treatment groups of rabbits. Loss of the righting reflex occurred at 48 ± 22 s in group 2 and at 34 ± 27 s in group 3. Loss of the ear pinch response and pedal withdrawal reflexes occurred at 88 ± 34 s and 94 ± 40 s, respectively, in group 2 and at 78 ± 32 and 78 ± 38 s in group 3. The times required for return of the ear pinch response and pedal withdrawal reflexes were 13.3 ± 1.39 and 11.8 ± 1.10 min, respectively, in group 2 and 30.7 ± 2.90 and 29.2 ± 2.90 min in group 3. The time until return of the righting reflex was 35 ± 18 min in group 2 and 58 ± 24 min in group 3. In general, recovery from anesthesia was fast and uneventful in both groups once sternal recumbency (righting reflex, positive) was achieved, although 3 animals in group 3 demonstrated increased agitation, denoted by twitchy and sudden movements.

Cortisol and corticosterone levels. For group 3 rabbits, which received propofol–ketamine, serum cortisol levels (Table 1) were increased at 5, 10, 30, and 60 min ($P < 0.001$ in all cases) when compared with the other 2 groups at the corresponding time points. In addition, serum corticosterone levels (Table 1) were elevated from 5 to 60 min in group 3 ($P < 0.001$ in all cases) when compared with groups 1 and 2. Cortisol and corticosterone levels did not differ between groups 1 and 2 throughout the study.

Heart and respiratory rates. Heart rates (Table 2) between 5 and 60 min in group 2 were decreased ($P < 0.05$) compared with those at baseline and with those of groups 1 and 3 at the same time points. Similarly, respiratory rate (Table 2) was decreased in group 2 from 5 to 60 min after injection ($P < 0.001$) and in group 3 from 5 to 30 min when compared with group 1 ($P < 0.01$).

Rectal temperature. Mean rectal temperature (38.2 ± 0.7 °C) did not change significantly in either group during anesthesia.

Discussion

Some anesthetic protocols may modify adrenal function. Effective methods for modulating the stress response are desirable to minimize secondary effects during the perioperative period. The results of this study reveal a consistent response of the corticoadrenal gland in terms of glucocorticoid secretion after the induction of anesthesia with ketamine and propofol. However, serum glucocorticoid concentrations did not change significantly after dexmedetomidine–propofol anesthesia. Some authors have reported that glucocorticoid levels increase after propofol anesthesia in rats.[9] Recent results suggest that propofol has a stimulatory effect on serum cortisol—but not corticosterone—concentrations in rabbits. This scenario indicates differences in the patterns of propofol stimulation during the release of different glucocorticoids in rabbits.[14] According to our current results, dexmedetomidine might have inhibited propofol-induced increases in serum cortisol. The stress-alleviating properties of this drug have been well documented.[5,28] Surgical stress response might be attenuated by sympatholytic activities mediated by dexmedetomidine.[29] Given that α2 adrenoceptor-mediated actions and perioperative stressors are related, dexmedetomidine may influence the hypothalamic–pituitary–corticoadrenal axis during anesthesia and recovery.[19] Previous studies have shown correlations between dexmedetomidine and decreased glucocorticoid levels in dogs, rabbits, and humans.[6,12,15,21,28] Medetomidine decreased plasma cortisol levels dogs experiencing stressful events, such as general anesthesia followed by abdominal surgery.[27] The administration of propofol–dexmedetomidine does not significantly alter serum glucocorticoid levels in humans.[11]

In contrast to effects of propofol–dexmedetomidine, rabbits anesthetized with propofol–ketamine showed an increase in

Table 1. Serum concentrations (ng/mL) of cortisol and corticosterone in rabbits (n = 10 per group)

	Cortisol			Corticosterone		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
0 min	2.60 ± 0.39	2.88 ± 0.45	2.67 ± 0.42	7.25 ± 1.01	7.63 ± 0.89	7.27 ± 1.07
5 min	2.92 ± 0.38	5.54 ± 0.71	19.67 ± 3.45[a]	7.49 ± 0.86	3.89 ± 0.43	21.62 ± 3.15[a]
10 min	2.83 ± 0.39	4.68 ± 0.69	19.31 ± 2.78[a]	7.03 ± 0.79	5.82 ± 0.61	17.82 ± 2.21[a]
30 min	2.69 ± 0.37	3.94 ± 0.77	18.91 ± 2.60[a]	6.71 ± 0.91	8.77 ± 0.99	23.57 ± 3.12[a]
60 min	2.46 ± 0.30	3.44 ± 0.51	20.27 ± 2.35[a]	6.75 ± 0.97	7.95 ± 0.95	25.57 ± 3.45[a]
24 h	2.67 ± 0.45	3.11 ± 0.44	3.03 ± 0.41	7.23 ± 0.67	8.99 ± 0.82	7.42 ± 1.33

Group 1 received normal saline intravenously; group 2 received propofol (3 mg/kg IV) and dexmedetomidine (0.35 mg/kg IM); and group 3 received propofol (3 mg/kg IV) and ketamine (1 mg/kg IV). All values are expressed as mean ± SD.
[a]Value is significantly ($P < 0.001$) different from the baseline value (0 min) and compared with groups 1 and 2 at the same time point.

Table 2. Heart rate (beats/min) and respiratory rate (breaths per minute) rabbits (n = 10 per group)

	Heart rate			Respiratory rate		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
0 min	262.4 ± 7.28	259.2 ± 8.28	270.2 ± 9.22	126.0 ± 6.19	121.6 ± 5.36	115.2 ± 4.23
5 min	274.6 ± 7.65	113.6 ± 10.57[c]	263.0 ± 8.77[e]	124.3 ± 5.86	62.2 ± 6.58[c]	79.2 ± 6.38[c]
10 min	273.8 ± 7.42	95.0 ± 5.81[c]	261.8 ± 8.63[e]	123.5 ± 5.22	50.0 ± 6.20[c]	69.8 ± 5.48[c]
30 min	269 ± 6.39	103.4 ± 6.26[c]	256.6 ± 11.05[e]	124.0 ± 4.84	55.2 ± 6.77[c]	94.2 ± 8.61[b]
60 min	264 ± 6.23	225.8 ± 18.58[a]	260.2 ± 10.66[d]	126.0 ± 4.77	73.0 ± 4.29[c]	115.8 ± 7.83
24 h	278 ± 6.75	268.0 ± 3.91	268.4 ± 6.22	128.0 ± 5.22	116.2 ± 6.29	118.0 ± 3.84

Group 1 received normal saline intravenously; group 2 received propofol (3 mg/kg IV) and dexmedetomidine (0.35 mg/kg IM); and group 3 received propofol (3 mg/kg IV) and ketamine (1 mg/kg IV). All values are expressed as mean ± SD.
[a–c]Value is significantly ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$) from the baseline values (0 min) and compared with group 1 at the same time point.
[d,e]Value is significantly ([d]$P < 0.05$, [e]$P < 0.001$) different compared with group 2.

serum glucocorticoid concentrations. This increase probably reflects both propofol- and ketamine-associated stimulatory effects on corticoadrenal function. In addition to the stimulatory effect of propofol, ketamine has been shown to increase serum glucocorticoid concentrations in dogs, monkeys, and humans.[1,18,25] Propofol–ketamine administration increased cortisol concentrations both during and after surgery in humans.[7] Both propofol- and ketamine-associated stimulatory effects are consistent with the results we obtained in rabbits treated with this combination.

A potential limitation of the current study is that it was not designed to test the effects of these anesthetic combinations when followed by surgery, which is a potently stressful event. Therefore, further studies are needed to clarify the effects of these anesthetic combinations on serum glucocorticoid levels in rabbits experiencing stressful events, such as surgical stimulus.

In our study, respiratory rate decreased in groups 2 and 3, and heart rate was reduced concurrently in group 2. Both propofol and dexmedetomidine have been reported to cause cardiovascular and respiratory depression in rabbits,[8,15] probably due to sympathetic inhibition.[13,26] The decrease in heart rate was less pronounced when propofol was administered with ketamine; this effect probably reflects ketamine-associated stimulatory effects on the sympathetic system.[4,10] Therefore the addition of ketamine might have counteracted the inhibitory effect of propofol, thus establishing appropriate cardiovascular stability in our rabbits.

On the basis of the results obtained in this study, we conclude that propofol–ketamine anesthesia in rabbits provides suitable cardiorespiratory stability but enhances glucocorticoid secretion more than dexmedetomidine–propofol anesthesia. When designing experiments, researchers should consider effects on glucocorticoid levels in anesthetized rabbits to minimize their stress response to surgery and to avoid erroneous interpretation of data collected from these animals.

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