Comparative studies on blood ACTH, cortisol, adrenaline, insulin and glucose in ovariohysterectomized cats anesthetized with isoflurane alone or combined with butorphanol and meloxicam

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ABSTRACT

A study has been carried out with fourteen healthy mature cats in order to determine the effect of two anesthetic protocols on the concentrations of blood adrenocorticotropic hormone (ACTH), cortisol, insulin and glucose. The animals were randomly allocated in two experimental groups (n=7). The premedication in the first group (group In) was made with acepromazine, intramuscularly and in the second group (group MM) - with acepromazine and butorphanol (intramuscularly) and meloxicam (subcutaneously). Anesthesia was induced with propofol, intravenously, fifteen minutes after premedication. The anesthesia was maintained with isoflurane. Ovariohysterectomy was performed upon occurrence of deep anesthesia after the 30th minute. Blood specimens were obtained at 0, 30, 60, 120 min and 24 h. Pronounced decrease in blood ACTH, cortisol, adrenaline in group In was determined 120 min after the end of surgery. In contrast, the release of stress hormones in group MM was inhibited immediately after the application of the anesthetics, as the lowest concentrations were registered at the 60th min. Significant hyperglycemia together with hyperinsulinemia in both groups was established at the 120th min from the beginning of the anesthesia. Anesthesia with isoflurane and propofol in cats decreased blood concentrations of stress hormones immediately after the end of the surgical intervention, whereas the multimodal anesthesia, including opioid and non-steroidal anti-inflammatory drugs (NSAID) led to reduction of the stress intervention, whereas the multimodal anesthesia, including opioid and non-steroidal anti-inflammatory drugs (NSAID) led to reduction of the stress response. Furthermore, the different animal species exhibit a different endocrine response to the same anesthetic agents and methods [14, 20-22, 27, 28]. The endocrine changes during and after anesthesia were studied in different animal species [1, 2, 7, 11, 13, 17, 28], but the investigations in cats are limited [3, 14, 15].

Introduction

In previous studies it was found that the endocrine response depended on the anesthesia, the combinations of various anesthetic agents, surgical procedures, level of pain, etc. Furthermore, the different animal species exhibit a different endocrine response to the same anesthetic agents and methods [14, 20-22, 27, 28]. The endocrine changes during and after anesthesia were studied in different animal species [1, 2, 7, 11, 13, 17, 28], but the investigations in cats are limited [3, 14, 15].

Keywords: Anesthesia, cats, adrenaline, cortisol, adrenocorticotropic hormone, insulin, glucose, isoflurane, butorphanol, meloxicam.

RÉSUMÉ

Une étude a été réalisée sur quatorze chattes matures saines afin de déterminer l’effet de deux protocoles anesthésiques sur les concentrations sanguines en adrénaline, hormone corticotrope (ACTH), cortisol, insuline et glucose chez les chattes ovario-hystérectomisées anesthésiées avec de l’isoflurane seul ou en combinaison avec le butorphanol et le meloxicam.

Animals undergoing ovariohysterectomy require reliable intraoperative and postoperative control of pain. This is very important particularly for cats, with regard to their proper recovery after the surgical intervention. Opioids, such as butorphanol, provide an effective analgesia, although of short duration [6, 26].

NSAIDs, such as meloxicam, produce effective analgesia for a longer period when administered preoperatively, but their use in cats is less frequent [6, 25]. The combination of opioids and non-steroidal drugs may result in a better analgesic effect, but could also cause changes in stress-related

Mots-clés : anesthésie, chats, adrénaline, cortisol, hormone corticotrope, insuline, glucose , isoflurane, butorphanol, meloxicam.
hormones, such as ACTH, cortisol, adrenaline and insulin [4, 30]. The information for the neurohormonal effects of these drugs in cats is limited [5].

The aim of the this study was to investigate the effects of two anesthetic protocols on plasma concentrations of ACTH, cortisol, adrenaline, insulin and serum blood glucose during ovariohysterectomy in cats.

Materials and methods

ANIMALS

Fourteen female cats at the age between 2 and 4 years, weighing 2.8 – 3.9 kg, mixed breed, were included in the study. Two weeks before the experiment, the animals were kept in the University Clinic for Small Animals at the Faculty of Veterinary Medicine, University of Forestry, Sofia. They were fed commercial dry food without limitation except for the 12-hour fasting period before the anesthesia and surgery. The water was restricted two hours before surgery.

Immediately prior to the experiment, the animals were examined and determined to be clinically healthy on the basis of the physical and blood laboratory examinations, including complete blood counts and total protein. All values were within normal physiological ranges.

ANESTHETIC PROTOCOLS

The cats were randomly allocated in two experimental groups (n=7 in each group). The premedication in the first group (group In) was made with acepromazine maleate 0.025 mg/kg (Vetranquil®, Ceva Santé Animale) intramuscularly, and the second group (group MM) was given acepromazine maleate 0.025 mg/kg (Vetranquil®, Ceva Santé Animale), butorphanol (Butomidor®, Richter Pharma)–0.4 mg/kg, intramuscularly and meloxicam (Loxicom®, Norbrook) - 0.3 mg/kg, subcutaneously. All animals were submitted to fluid therapy with sodium chloride 0.9 %, 10 ml/kg/h (Natrii chloridum®, Actavis) through a venous catheter 22 gauge (B.Braun) applied in v. cephalica antebrachii. Induction of anesthesia was made with propofol (Propofol®, B.Braun) at 2 ml/kg, intravenously, fifteen minutes after the premedication. Immediately after the application of the general anesthesia, the animals were intubated with a tube of a suitable size. The anesthesia was maintained with isoflurane (Forane®, Abbott) 2.5 vol. % in group In and 1.8 vol.% in group MM in 2.5 l/min oxygen flow by using semi-opened breathing circuit system type T/Y detail, Kuhn modification. The extubation was made 60 min later at manifestation of swallowing reflex.

SURGERY PROTOCOL

Ovariohysterectomy was performed through caudal median laparotomy. The average duration of the operation was between 8 and 10 min. Surgery started 30 minutes after the initiation of anesthesia at the surgical plane of anesthesia.

COLLECTION OF BLOOD SPECIMENS

Blood specimens were obtained from the jugular vein in sterile 2.0 ml syringes by 23 G needles at strictly determined intervals - at 0 min (before the application of the anesthetics) 30, 60, 120 min and 24 h from the beginning of the anesthesia. Immediately after collection of the specimens, 1.5 ml of each sample was put into a sterile micro vacutainer, containing heparin and centrifuged for 15 min at room temperature for hormonal analysis. The plasma specimens were stored at -22 °C for 27 days, prior to determination of the hormone concentration.

The rest 0.5 ml aliquot was put in sterile micro vacutainers without anticoagulant for blood glucose analysis. The specimens were incubated for two hours at 37°C to form a clot and after centrifugation the obtained serum was immediately analyzed to determine the blood glucose concentrations.

ANALYTICAL METHODS OF STUDY

Cortisol – by ADVIA Centaur®Cortisol test, Bayer Diagnostics. The examination was made by IMMULITE® 2000/2500 apparatus, Siemens, Germany. Enzyme Conjugate - Cortisol conjugated to horseradish peroxidase. The inter-assay CVs ranged between 6.5% and 7.7%. The limit of quantification was 6.9 nmol/l; Insulin – by specific Insulin ELISA test, Mercodia, Sweden. The test used feline insulin as calibration solution. The limit of quantification was 9.2 ng/L. The inter-assay CVs ranged between 6.7% and 12.5%; Adrenaline – by specific enzyme test Adrenaline - ELISA DRG Adrenaline ELISA, EIA_4306 – for quantitative determination of adrenalin in animal plasma, DRG Instruments GmbH, Germany. Enzyme Conjugate - anti-rabbit IgG conjugated with peroxidise. The limit of quantification for plasma was 60 pmol/L. The inter-assay CVs ranged between 13.2% and 15.4%; Adrenocorticotropic hormone – by specific enzyme test ACTH ELISA-test, 21-ACTHU-E01, ALPCO Diagnostics, USA, using lyophilized feline ACTH as a calibration solution. The limit of quantification was 0.1 pmol/L. The inter-assay CVs ranged between 5.8 % and 6.2 %.

The hormonal assays of adrenaline, ACTH and insulin were carried out by ELISA Reader Microplate Reader, France.

Blood glucose was quantitated by oxidase enzyme test (Spinreact, Glucose TR, Spain) on Biochemical Analysis System Hitachi 7070, Japan. The inter-assay CVs ranged between 1.58 % and 1.50 %. The limit of quantification was 0.05 mmol/L.
STATISTICAL ANALYSIS

All data were expressed as median and range. Differences between the two groups were analyzed using one way analysis of variance (ANOVA) and the least-significant difference (LSD) post hoc test at a level of significance 0.05.

The study was approved by the Committee on Animal Ethics of the National Veterinary Service in Bulgaria.

RESULTS

When comparing the obtained values of the examined analytes, no significant differences in the initial concentrations (0 min) between the two groups were determined.

A significant hyperglycemia was observed in group In at the 120th min – 11.2 (6.7-15.2) mmol/L (p<0.001) together with increased insulin concentrations in the same period – 27.7 (24.9-57.5) pmol/L, (p<0.01). The cortisol concentrations were increased at the 30th min and 60th min, but at the 120th min the concentrations were much lower than the initial ones – 171.9 (163.1-222.1) nmol/L. The lowest adrenaline concentrations were determined at the 120th min – 92.7 (90.5-93.3) pmol/L as compared to the initial values.

A significant decrease of the ACTH concentration in group In, compared to the initial values, was determined at the 120th min – 1.4 (0.5-2.1) pmol/L (p<0.05). By the 24th hour the concentration of ACTH in this group was similar to the baseline values (Table I).

The blood glucose concentrations in group MM were unchanged by the 30th and 60th min. A pronounced hyperglycemia was determined at the 120th min - 10.5 (8.5-12.1) mmol/l; (p<0.001). The concentrations were significantly lower at the 24th h as compared to the initially measured values - 3.8 (3.0-4.9) mmol/l; (p<0.05). The insulin concentrations were insignificantly increased at the 30th, 60th and 120th min from the beginning of the anesthesia and lower – 16.3 (11.9-17.9) pmol/L by the 24th hour vs. 0 min. In group MM the cortisol concentration was significantly higher at min 120th as compared to 0 min – 216.6 (131.6-284.5) nmol/L; (p<0.01). The changes in adrenaline concentrations in group MM were without statistical significance, as the lowest concentrations – 91.8 (93.9-125.6) pmol/L at the 24th hour as compared to the initial value.

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<th>Glucose (mmol/L)</th>
<th>Insulin (pmol/L)</th>
<th>Cortisol (mmol/L)</th>
<th>Adrenaline (pmol/L)</th>
<th>ACTH (pmol/L)</th>
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<td>0 min</td>
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<td>Group In</td>
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<td>5.4 (4.6-6.2)</td>
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<td>Group MM</td>
<td>5.4 (4.8-5.6)</td>
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<td>17.3 (13.7-19.5)</td>
<td>18.3 (14.0-27.1)</td>
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<td>21.5 (13.7-29.9)</td>
<td>29.3 (20.2-45.3)</td>
<td>28.2 (21.0-59.9)</td>
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<td>Group In</td>
<td>235.6 (227.1-248.5)</td>
<td>276.2 (174.9-335.8)</td>
<td>280.0 (165.8-331.4)</td>
<td>171.9 (163.1-222.1)</td>
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<td>166.9 (136.6-278.7)</td>
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<td>91.8 (87.6-95.0)</td>
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<td>1.7 (1.3-2.1)</td>
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<td>0.9 (0.2-1.3)</td>
<td>1.4 (1.3-1.7)</td>
<td>2.4 (1.8-2.5)</td>
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* p<0.05; p<0.01; p<0.001 vs. hour 0 within a group; # p<0.05; ## p<0.01; ### p<0.001 between groups at each time interval

Table I: Changes in the concentrations of blood glucose, insulin, cortisol, adrenaline and ACTH in group In and group MM

Significant decrease of ACTH concentration in group MM was determined by the 60th min – 0.9 (0.2-1.3) pmol/L; (p<0.01), however by the 24th hour the concentrations were statistically significantly higher - 2.4 (1.8-2.5); (p<0.001), in comparison with initial ones (Table I).

The analysis of blood glucose concentrations of both groups demonstrated statistically significantly higher values in group In only by the 24th hour as compared to group MM – 5.2 (4.2-6.5) mmol/L and 3.8 (3.0-4.9) mmol/L, respectively. Blood plasma insulin differed substantially between the groups on min 30 (p<0.05). Cortisol concentrations in group In were statistically significantly higher by min 30: 276.2 (174.9-335.8) nmol/L, (p<0.05) and min 60: 280.0 (165.8-331.4) nmol/L; (p<0.001) compared to group MM. A significantly lower adrenaline concentration in group In – 93.7 (92.2-120.7) pmol/L vs. group MM – 122.3 (93.9-125.6) was established only 24 hours after the anesthesia (p<0.05). Blood plasma ACTH of group In turned out to be considerably elevated (p<0.001) compared to group MM by the 60th min - 2.1(1.6-2.4) pmol/L and 0.9 (0.2-1.3) pmol/L. Unlike that, by the 24th hour, ACTH concentrations of the group anesthetized with isoflurane alone - 1.6 (1.4-2.4) pmol/L - were lower (p<0.05) that respective values of group MM - 2.4 (1.8-2.5) pmol/L (Table I).

Discussion

General anesthesia with volatile agents could suppress the nociception of surgical stimuli, but could not entirely inhibit the hypothalamic response, even during surgical plane of anesthesia. As a result, blood concentrations of ACTH, cortisol and adrenaline persist elevated due to pituitary gland stimulation [7, 9, 17, 31].

So far, available reports show that after the surgical stimuli are no longer effective, blood ACTH, cortisol and adrenaline decrease in response to sympatico-adrenal system suppression [18, 19].

Our study demonstrated that blood ACTH, cortisol and adrenaline concentrations in the group with volatile anesthesia only, were increased immediately after the application of anesthetics and persisted higher until ovariohysterectomy end. Only by the 2nd hour after the beginning of anesthesia (min 120), blood concentrations of assayed hormones were reduced.

The approaches to pain management in cats are entirely different from those used in the past. It is reported that the former beliefs about opioid-induced mania or higher NSAID toxicity in this species are not justified [25, 29].

The effects of opioids are species-dependent. In rats, they stimulate ACTH and cortisol release while in men, these hormones are inhibited [24]. In horses, butorphanol did not alter blood plasma cortisol, either with or without surgery [8, 16].

The independent pre-operative application of butorphanol in cats did not provide adequate analgesia; moreover, analgesia duration is relatively short. As a result, blood concentrations of adrenaline, cortisol and glucose could be elevated [25]. On the contrary, combinations of butorphanol with α₂-agonists, cataleptics, phenothiazine derivatives and NSAIDs reduce blood concentrations of stress hormones [25].

Meloxicam is successfully used in cats for control of pain and stress regardless of the fact that feline metabolism is rather different from that of other species [25]. The preoperative application of such drugs alleviates systemic stress response [12] via reduction of plasma cortisol and adrenaline concentrations [5, 10, 23].

In group MM, blood ACTH, cortisol and adrenaline were decreased immediately after application of anesthetics, with lower concentrations by the 60th min no matter the surgery.

Blood glucose changed in a similar manner after both anesthetic protocols, although cats from group In had lower intraoperative blood insulin, most probably resulting from increased catecholamine concentrations.

In conclusion, the results from the present study demonstrated that endocrine response was modulated by the type of the anesthetic protocol applied for the same surgical intervention.

Cats anesthetized with isoflurane only exhibited lower blood concentrations of stress hormones after the removal of surgical stimuli. The multimodal anesthesia resulted in lower blood ACTH, cortisol and adrenaline not considering the surgery. The preoperative co-administration of opioid and NSAID provide a stress-free period and could be used for control of pain in ovariohysterectomized cats.

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REFERENCES


