

# Effect of Xylazine-Ketamine Anesthesia on Blood ACTH, Cortisol, Adrenaline, Insulin and Glucose in Ovariectomized Cats

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Received: May 3, 2015 / Accepted: May 6, 2015 / Published: May 30, 2015.

**Abstract:** A study has been carried out with fourteen healthy mature cats in order to determine the effect of a xylazine-ketamine anesthetic protocol (group X/K) on blood concentrations of adrenaline, ACTH (adrenocorticotropic hormone), cortisol, insulin and glucose in comparison with a control group (group K—no anesthesia and surgery). The animals were randomly allocated in two groups ( $n = 7$ ). The premedication in the experimental group was made with xylazine 2 mg/kg intramuscularly. Induction and maintenance of anesthesia were made with ketamine 10 mg/kg intramuscularly. Ovariectomy was performed upon occurrence of deep anesthesia. Blood specimens were obtained at 0, 30, 60, 120 min and 24 h from the two groups. Pronounced decrease in blood ACTH, cortisol, and adrenaline in group X/K was determined by the 30 min. Significant hyperglycemia together with hyperinsulinemia in the X/K group was established at the 120 min from the beginning of the anesthesia. Anesthesia with xylazine and ketamine led to reduction of the blood levels of stress hormones immediately after the beginning of anesthesia and caused a remarkable hyperglycemia with hyperinsulinemia.

**Key words:** Anesthesia, cats, adrenaline, cortisol, ACTH, insulin.

## 1. Introduction

A number of previous studies have found that the endocrine response depended on anesthesia, the combinations of various anesthetic agents, surgical procedures, level of pain, etc. The endocrine changes during and after anesthesia were studied in different animal species, but the investigations in cats are limited [1-3].

The combination of xylazine and ketamine is often used in veterinary practice for different operations with short duration. This anesthetic protocol is easy to apply and relatively inexpensive. The side effects of  $\alpha_2$ -adrenoreceptors are usually associated with the cardiovascular system [4], but their neuroendocrine effects in cats are not fully understood. The

$\alpha_2$ -agonists strongly interfere with the neuroendocrine system and could be a predisposing cause for different diseases such as diabetes mellitus, Cushing disease, etc. [2].

The dissociative anesthetics have also endocrine effects. They act on receptor systems involved in the physiological stress response [5]. There is therefore a certain analogy between the endocrine effects of analgesics and the stress response.

The combination of  $\alpha_2$ -adrenoreceptor agonists and dissociative anesthetic agents provides a better analgesic effect, but could also cause changes in stress-related hormones, such as ACTH, cortisol, adrenaline and insulin [1, 4, 6]. The information for the neurohormonal effects of this anesthetic protocol in cats is limited [3].

The aim of the study was to investigate the effects of xylazine-ketamine anesthesia on plasma concentrations of ACTH, cortisol, adrenaline, insulin

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and serum blood glucose during ovariectomy in cats in comparison with a control group not submitted to anesthesia and surgery.

## **2. Materials and Methods**

### *2.1 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 h 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.

The cats were randomly allocated in two groups—control (K) and experimental (X/K) group (n = 7 in each group). The premedication in the experimental group was made with xylazine hydrochloride 2 mg/kg (Xylasin<sup>®</sup>, Alfazan-Turkey) intramuscularly. All animals were submitted to fluid therapy with sodium chloride 0.9%, 10 mL/kg/h (Natrii chloridum<sup>®</sup>, Actavis) through a venous catheter 22 gauge (B. Braun) applied in v. cephalica antebrachii. Induction of anesthesia was made with ketamine hydrochloride, (Ketamin, Intervet-Holand) 10 mg/kg body weight intravenously, five minutes after the premedication. Immediately after the application of the general anesthesia, the animals were intubated with a tube of a suitable size. The oxygen flow was 2.5 L/min by using semi-opened breathing circuit system type T/Y detail, Kuhn modification. The extubation was made after manifestation of swallowing reflex. The anesthetics were not given to the animals from control group.

### *2.2 Surgery Protocol*

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

### *2.3 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 concentrations.

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.

### *2.4 Analytical Methods of Study*

Cortisol—by ADVIA Centaur<sup>®</sup> Cortisol test, Bayer Diagnostics. The examination was made by IMMULITE<sup>®</sup> 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 adrenaline in animal plasma, DRG Instruments GmbH,

Germany. Enzyme Conjugate-anti-rabbit IgG conjugated with peroxidase. The limit of quantification for plasma was 60 pmol/L. The inter-assay CVs ranged between 13.2% and 15.4%; Adrenocorticotrophic 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 7,070, Japan. The inter-assay CVs ranged between 1.58% and 1.50%. The limit of quantification was 0.05 mmol/L.

### 2.5 Statistical Analysis

All data were expressed as mean and standard deviation (mean  $\pm$  SD). Differences between the two groups were analyzed using one way ANOVA (analysis of variance) 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 at the National Veterinary Service in Bulgaria.

## 3. 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 X/K at the 30 min ( $9.3 \pm 3.1$  mmol/L), 60 min ( $15.3 \pm 2.4$  mmol/L) and 120 min— $18.1 \pm 1.3$  mmol/L ( $p < 0.001$ ) together with increased insulin concentrations at the same periods—30 min— $49.2 \pm 11.5$  pmol/L ( $p < 0.01$ ), 60 min— $68.0 \pm 23.4$  pmol/L and 120 min— $93.3 \pm 23.9$  pmol/L ( $P < 0.001$ ).

The cortisol concentrations were decreased at the 30 min— $154.5 \pm 19.5$  nmol/L ( $P < 0.001$ ) and its values were unchanged by the 60 min and 120 min. By the 24 h the concentration of cortisol was similar to the baseline values. The lowest adrenaline concentrations were determined at the 60 min— $89.5 \pm 2.0$  pmol/L as compared to the initial values.

A significant decrease of the ACTH concentration in group X/K, compared to the initial values, was determined at the 30 min— $0.9 \pm 0.6$  pmol/L ( $P < 0.01$ ). By the 60 min and 120 min the concentrations of ACTH in this group were significant lower— $0.4 \pm 0.2$  ( $P < 0.001$ ) and  $0.2 \pm 0.04$  ( $P < 0.001$ ). By the 24 h the concentration of ACTH was similar to the baseline values (Table 1).

The blood glucose and insulin concentrations in control group were unchanged. A significant increase of the cortisol concentration in group K, compared to the initial values, was determined at the 24 h— $338.4 \pm 4.3$  nmol/L ( $P < 0.01$ ). The adrenaline concentrations were increased significantly at the 30 min— $103.8 \pm 1.5$  pmol/L ( $P < 0.01$ ), but at the 60 min and 120min they were similar to the baseline values. Unlike that, by the 24 h, adrenaline concentrations of group K were higher than initial values— $121.3 \pm 1.0$  pmol/L ( $P < 0.01$ ).

Significant increase of ACTH concentration in group K was determined by the 30 min— $2.2 \pm 0.2$  pmol/L ( $P < 0.05$ ) and 24 h— $2.4 \pm 0.1$  pmol/L ( $P < 0.001$ ), however by the 60 min the concentrations were statistically significantly lower— $1.67 \pm 0.2$  pmol/L ( $P < 0.01$ ), in comparison with initial ones.

The analysis of blood glucose concentrations of both groups demonstrated statistically significantly higher values in group X/K by the 30 min ( $9.3 \pm 3.1$  mmol/L), 60 min ( $15.3 \pm 2.4$  mmol/L) and 120 min ( $18.1 \pm 1.3$  mmol/L),  $P < 0.001$  as compared to group K.

Blood plasma insulin differed significantly between the groups on all periods of examination.

Cortisol concentrations in group X/K were

statistically significantly lower by min 30:  $154.5 \pm 19.5$  nmol/L ( $P < 0.05$ ), min 60:  $159.0 \pm 5.4$  nmol/L ( $P < 0.001$ ) and hour 24:  $233.0 \pm 43.8$  ( $P < 0.01$ ) compared to group K. A significantly lower adrenaline concentration in group X/K vs. group K was established at 30 min— $92.0 \pm 0.7$  pmol/L ( $P < 0.05$ ) and 24 h after the anesthesia— $95.9 \pm 6.1$  ( $P < 0.001$ ). Blood plasma ACTH of group X/K turned out to be considerably decreased ( $P < 0.001$ ) compared to group K by the 30 min, 60 min and 120 min. By the 24 h, ACTH concentrations of the group anesthetized with xylazine and ketamine— $1.8 \pm 0.4$  pmol/L—were lower ( $P < 0.01$ ) than respective values of group K— $2.4 \pm 0.1$  (1.8-2.5) pmol/L (Table 1).

#### 4. Discussion

Alpha<sub>2</sub>—agonists are known to inhibit the sympathetic nervous system via their influence on α<sub>2</sub>—adrenoreceptors causing decrease in circulating catecholamine concentrations [7, 8], either with or without surgery [9] including in cats [2, 3]. On the other hand, being a NMDA antagonist, ketamine could stimulate or inhibit sympathetic activity [10, 11].

The authors have shown that the xylazine/ketamine combination resulted in an insignificant reduction of blood adrenaline immediately after anesthetic drug application which persisted until the end of the experiment.

Alpha<sub>2</sub>—adrenoreceptor agonists inhibit cortisol secretion, but it is still disputable whether these effects are mediated only via α<sub>2</sub>—receptors or other receptors are also involved. Recent reports suggest that imidazoline receptors play probably a role in the inhibition of cortisol secretion [2]. An in vitro study demonstrates that imidazoline α<sub>2</sub>—adrenergic agents medetomidine, detomidine and atipamezole inhibit cortisol secretion in porcine adrenocortical cells [12]. As medetomidine and detomidine are selective α<sub>2</sub>—adrenoreceptor agonists, and atipamezole—a selective α<sub>2</sub>—adrenoreceptor antagonist, their effects could not be mediated via influence on α<sub>2</sub>—adrenoreceptors only [8, 13]. Therefore, the secretion of ACTH and cortisol is probably mediated through imidazoline receptors as well.

In rats, xylazine and ketamine co-administration results in reduction of blood ACTH and corticosterone

**Table 1** Changes in the concentrations of blood glucose, insulin, cortisol, adrenaline and ACTH in group X/K and group K.

	0 min	30 min	60 min	120 min	24 h
Glucose (mmol/L)	$5.2 \pm 0.7$	$9.3 \pm 3.1$	$15.3 \pm 2.4$	$18.1 \pm 1.3$	$4.2 \pm 1.6$
Group X/K		***###	***###	***###	
Group K	$4.4 \pm 0.9$	$4.5 \pm 0.9$	$4.4 \pm 1.2$	$5.0 \pm 0.9$	$5.1 \pm 0.9$
Insulin (pmol/L)	$19.4 \pm 5.6$	$49.2 \pm 11.5$	$68.0 \pm 23.4$	$93.3 \pm 23.9$	$21.2 \pm 5.1$
Group X/K		#####	#####	#####	##
Group K	$21.2 \pm 3.4$	$20.7 \pm 4.8$	$18.4 \pm 0.3$	$22.1 \pm 3.5$	$13.3 \pm 0.8$
Cortisol (nmol/L)	$254.1 \pm 75.3$	$154.5 \pm 19.5$	$159.0 \pm 5.4$	$158.3 \pm 9.9$	$233.0 \pm 43.8$
Group X/K		***#	**###	***	##
Group K	$221.2 \pm 65.1$	$237.5 \pm 88.5$	$279.2 \pm 5.1$	$211.9 \pm 62.9$	$338.4 \pm 4.3$
					**
Adrenaline (pmol/L)	$104.0 \pm 13.7$	$92.0 \pm 0.7$	$89.5 \pm 2.0$	$92.5 \pm 8.2$	$95.9 \pm 6.1$
Group X/K		#			###
Group K	$94.3 \pm 2.1$	$103.8 \pm 1.5$	$93.5 \pm 1.1$	$94.1 \pm 0.8$	$121.3 \pm 1.0$
		*			**
ACTH (pmol/L)	$1.7 \pm 0.6$	$0.9 \pm 0.6$	$0.4 \pm 0.2$	$0.2 \pm 0.04$	$1.8 \pm 0.4$
Group X/K		**###	***###	***###	##
Group K	$1.9 \pm 0.2$	$2.2 \pm 0.2$	$1.67 \pm 0.2$	$2.0 \pm 0.2$	$2.4 \pm 0.1$
		*	**		***

[14]. The investigations on ketamine effects demonstrate that depending on the dose, plasma cortisol and ACTH could either increase or remain unchanged after its independent application [15, 16].

In the authors' experiment, blood ACTH and cortisol were statistically significantly reduced immediately after the application of anesthetics and this depression persisted in the next study periods. In control group of cats, the concentrations of both hormones were higher than the initial ones, which could be explained by the physiological stress due to manipulation of animals [17].

Studies performed so far affirm that the mechanism of hyperglycemia after application of  $\alpha_2$ -agonists was related to inhibition of insulin secretion via the agonistic effects on  $\alpha_2$ -receptors in pancreatic beta cells, but the effects of ketamine on blood insulin concentrations are variable. According to Maroto et al.[18], blood glucose in dogs is regulated by two opposing mechanisms involving  $\alpha$ - and  $\beta$ -adrenoceptor mediated effects. In rabbits, low doses of ketamine were found to have no effects on blood glucose, higher doses result in a substantial  $\alpha_2$  receptor mediated hyperglycemia and surprisingly, even higher doses induce hypoglycemia mediated via opioid and  $\beta$ -adrenergic receptors, evident only after blockage of  $\alpha_2$  receptors with the antagonist yohimbine [19].

In the group of cats with xylazine/ketamine anesthesia, a considerable hyperglycemia was observed immediately after anesthetic drug application. Blood glucose increased until the 2 h on the background of a progressive hyperinsulinemia and decreased plasma ACTH, adrenaline and cortisol.

The established hyperglycemia and hyperinsulinemia in the anesthetized group was most probably due to the interaction of  $\alpha_2$ -agonists and cataleptics and their concurrent effect on pancreatic  $\beta$ -adrenergic receptors.

The observed changes in blood cortisol and adrenaline in control cats were obviously too insignificant to alter blood glucose levels, which

varied within the physiological range at all sampling intervals.

## 5. Conclusions

The anesthetic drug combination of xylazine and ketamine induces a substantial hyperglycemia at the background of hyperinsulinemia, persisting for more than 120 min. The blood concentrations of stress hormones—ACTH, cortisol and adrenaline—decreased immediately after the application of anesthetics. The changes observed in the control group were specific for manipulation-induced physiological stress.

Xylazine-ketamine anesthesia is easy and appropriate for use in veterinary practice, ensuring systemic stress response inhibition for at least two hours, which is enough for performing medium-duration surgery. The use of tested anesthetics should be avoided in cats with abnormal glucose metabolism due to the occurring significant and prolonged hyperglycemia.

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