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Molecular and Cellular Biochemistry
An International Journal for Chemical Biology in Health and Disease

ISSN 0300-8177

Mol Cell Biochem
DOI 10.1007/s11010-020-03838-z

Springer

Molecular and Cellular Biochemistry
https://doi.org/10.1007/s11010-020-03838-z

The comparison of the effects of ketamine and etomidate on cardiodynamics, biochemical and oxidative stress parameters in Wistar male rats

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Received: 19 May 2020 / Accepted: 11 July 2020

Abstract

It is well known the use of ketamine and etomidate in clinical practice; however, the difference in the systemic effects of these two anesthetic agents is still debatable. Thus, in the present study we aimed to compare their effects on heart, and other organs through estimation of cardiodynamics, biochemical and hematological parameters. Male Wistar rats were divided in 2 groups containing of 2 subgroups ($n=7$ in each subgroup, $n=28$ in total): (1) bolus injection of anesthetic ketamine (40 mg/kg b.w., i.p. $n=14$); (2) bolus injection of anesthetic etomidate (20 mg/kg b.w., i.p. $n=14$). The experiments were done in vitro in one subgroup of each group: cardiodynamic variables (dp/dt_{max}, dp/dt_{min}, heart rate), coronary flow, oxidative stress in coronary effluent and cardiac tissue homogenate, and in vivo in another subgroup: biochemical and hematological parameters, and oxidative stress in haemolysate. Significantly increased left ventricular contractility (dp/dt_{max}) and relaxation (dp/dt_{min}) were noticed in etomidate group. Creatinine (CREA), HDL cholesterol and folate were significantly higher in etomidate group, whereas amylase (AMY) and eosinophils in ketamine group. Our results suggested that ketamine has more antioxidant potential compared to etomidate, and etomidate has more favorable effects regarding cardiac performance.

Keywords Anesthetics · Ketamine · Etomidate · Cardiodynamics · Biochemical parameters · Hematological parameters · Oxidative stress · Rat

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Introduction

Ketamine and etomidate are intravenous anesthetic agents, especially important for their good anesthetics properties but not widely used because of their safety and various effects regarding hemodynamic function [1]. This is particularly important as we know that other agents such as thiopental and propofol exert vasodilatory and negative inotropic effects [2, 3]. However, their use is still debatable due to different benefits or side effects associated with both ketamine and etomidate, and this gap in knowledge should be overcome in animal models.

Ketamine is known as non-competitive *N*-methyl D-aspartate (NMDA) receptor antagonist, having dissociative, cognitive, psychotomimetic and peripheral side effects. On the other hand, its analgesic, anti-inflammatory and anti-depressant actions are also described [4]. Substantial body of knowledge indicates its stimulatory sympathomimetic and inhibitory parasympathetic effects on the heart, increasing heart rate and arterial pressure [5].

Published online: 26 July 2020

Also, ketamine may act directly on the myocardial cells and has dual action on the transmembrane potential of the Purkinje fibers. So, the main effects of ketamine in based on blockade of cardiac vagal nerves, increasing the levels of catecholamine and constriction of the alpha adrenoreceptor blood vessels with increased venous return to the heart [6]. Some authors demonstrated that it has a dual effect on the myocardium: a positive and a negative inotropic effect [7]. Positive effects are mainly due to increase in sympathetic activity [8, 9].

On the other hand, etomidate is an ultrashort-acting agent which in opposite to ketamine, induce only transient apnea with good cardiovascular stability in animals and humans [10, 11]. It also can cause nausea, vomiting, myoclonus, pain on injection and impairment of endocrine system function [12]. Cardiovascular stability is attributed to the stimulation of central adrenergic receptors, with a lack of myocardial depression and little change in the heart rate or blood pressure [13].

Induction doses of etomidate inhibit the 11-β hydroxylase enzyme and cause transient adrenal suppression that may negatively impact severely injured patients [10, 11]. Although ketamine has emerged as an alternative to etomidate for some conditions in emergency medicine, experience with ketamine in this setting is limited due to historical concerns about it increasing intracranial pressure, and evidence suggesting it has a direct myocardial depressant effect, which may lead to complications in critically ill patients with diminished physiologic reserve [10–13]. Even it is known that the ketamine has stronger cardio-depressant effect compared to etomidate, little is known how these two general anesthetics could influence on redox status and routine biochemical parameters after single administration. In that sense, it is important to confirm and explain clearly effects of these anesthetics on systemic response even after single use on animal model with potential clinical significance of these data.

We hypothesized that etomidate provides better overall performances and results in fewer adverse effects compared to ketamine. Although many studies have already been done on myocardium in different species, we aimed to give an overall insight into effects of these two anesthetics. It would improve the understanding of mechanisms of actions and most important it would help in choosing the better option in the specific clinical case. Measurements of cardiodynamic variables and oxidative stress in heart were done in vitro, whereas biochemical and hematological parameters were evaluated in vivo. In vitro studies were done to obtain the most accurate and direct effects on intrinsic myocardial contractility. In vivo experiments are influenced by concurrent changes such as preload, afterload, sympathetic activation, and baroreflex activity.

Material and methods

Ethical approval

All applicable international, national and institutional guidelines for the care and use of animals were followed. Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and European Directive for Welfare of Laboratory Animals No. 2010/63/EU. The ethics protocol of the current study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Belgrade, Serbia (No. 4196/2 from 08/10/2018).

Animals

Male *Wistar albino* rats (*Ratus norvegicus*), with a body weight of approximately 200–250 g and age of 6–8 weeks were used. The rats were housed in pairs in transparent Plexiglas cages with a wood-chip floor. Food and water were available ad libitum, and the ambient conditions were constant (temperature 21 ± 2 °C; humidity 55 ± 5%; 12 h light–dark cycle with the light period beginning at 07:30 a.m.). After a 1-week period of acclimatization, experimental procedures were begun.

Experimental protocol

Experimental animals were divided in 2 groups consisting of 2 subgroups each one, with 7 animals in each subgroup ($n = 28$ in total): (1) intraperitoneally injection of anesthetic ketamine (40 mg/kg b.w., i.p. $n = 14$) (*K* group [14], (2) intraperitoneally injection of anesthetic etomidate (20 mg/kg b.w., i.p. $n = 14$) (*E* group) [15]. The experiments were done in vivo and ex vivo: cardiodynamic variables [maximum and minimum rate of left ventricle pressure (dp/dt_{max} and dp/dt_{min}; systolic and diastolic left ventricle pressure (SLVP and DLVP; heart rate (HR], coronary flow (CF, and oxidative stress in coronary effluent, haemolysate and cardiac tissue homogenate superoxide anion radical (O_2^-) hydrogen peroxide (H_2O_2,), nitrites (NO_2^-), Index of lipid peroxidation measured as TBARS, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) and biochemical and hematological parameters.

Anesthetics were administered twice at intervals of 10 s until anesthetics took effect. After 15 min animals were sacrificed using a rat guillotine, and blood samples were taken. Anesthetics were given by intraperitoneally injection, in clinical equivalent doses, because of the good bioavailability after this route of administration [16].

Measurement of cardiodynamic variables according to the Langendorff technique

The hearts were quickly removed after anesthesia application and heparinization and retrograde perfused through the aorta in a Langendorff apparatus (Experimetria, Hungary) at a constant pressure of 70 cm H_2O. Normal Ca^{2+}-containing Krebs–Henseleit solution, equilibrated with 95% O_2/5% CO_2, was kept at 37 °C and used for perfusion. The Krebs–Henseleit solution contained 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose. A latex balloon tipped with a pressure transducer was inserted into the left ventricle via the left atrium for measurements of heart performance. The A/D signal was recorded using Labchart 7 software (ADInstruments, Shanghai, China) and stored in a computer. Using a sensor placed in the left ventricle of isolated rat heart, we registered and continuously recorded the following parameters of myocardial function: maximal rate of rise of left ventricular pressure (dp/dt_{max}), minimal rate of rise of left ventricular pressure (dp/dt_{min}), heart rate, and coronary flow. The experiment was started after the stabilization period at 70 cm H_2O coronary perfusion pressure.

Determination of oxidative stress parameters (superoxide anion radical, nitrites, hydrogen peroxide and index of lipid peroxidation) in coronary effluent

In coronary venous effluent, the following oxidative stress markers were measured: superoxide anion radical (O_2^-), nitrites (NO_2^-), hydrogen peroxide (H_2O_2), and lipid peroxidation index (thiobarbituric acid reactive substances, TBARS). These parameters were measured in the conditions of gasotransmitters production inhibition and (or) Ca^{2+} channel inhibition in propofol-anesthetized rats. Determination of O_2^- in the coronary venous effluent is based on the O_2 reaction with nitro tetrazolium blue (nitro blue tetrazolium, NBT), which makes nitroformase blue [17]. Measurement is performed at the wavelength of maximum absorbance $\lambda_{max} = 550$ nm. The spectrophotometric method for determining the amount of released nitrite [18] is an available and sufficiently reliable method for this assessment. Since, in the reaction with molecular oxygen: $NO + 1/2O_2 \rightarrow NO_2^-$, an equimolar amount of nitrite is produced, it can be highly assured that the amount of liberated nitrites in the coronary venous effluent represents the amount of NO released. The biochemical method is based on the use of the Griess-reagent, which makes a diazo complex with nitrites, and gives the purple color. Determination of H_2O_2 is based on the oxidation of phenol red by hydrogen peroxide reaction which is catalyzed by horse radish peroxidase, HRPO. This reaction gives a compound whose maximum absorbance is $\lambda_{max} = 610$ nm [19]. TBARS is determined indirectly through products of the lipid peroxidation reaction with thiobarbituric acid (thiobarbituric acid reactive substances). The values of TBARS in the coronary venous effluent can be determined spectrophotometrically [20]. This method is based on the reaction of one of these substances, malonildialdehyde (MDA) with thiobarbutyric acid (TBA).

Determination of antioxidative defense parameters in haemolysate and in the cardiac tissue homogenate

SOD activity was determined by the Beutler epinephrine method. 100 µL of epinephrine was added to the mixture of 100 µL of lysate and 1 mL of carbonate buffer. The measurement was performed spectrophotometrically at 470 nm [21]. CAT activity was determined after dilution of the lysate with distilled water in a ratio of 1:7 and the addition of ethanol in a ratio of 0.6:1. 50 µL of CAT buffer, 100 µL of sample and 1 mL of 10 mM H_2O_2 were placed in a tube and measured spectrophotometrically at 360 nm [22]. The activity of reduced glutathione molecule (GSH) was determined in the lysate and measured by the spectrophotometric method. This method is based on the reaction of glutathione oxidation with 5, 5-dithio-bis-6, 2-nitrobenzoic acid, DTNB [23].

The cardiac tissue was homogenized and centrifuged for 10 min at 20,000 × *g* (Eppendorf 5430 R). It was frozen at − 20 °C until the time of analysis. In cardiac tissue homogenate, we measured the activity of the following antioxidant defense parameters: superoxide dismutase, catalase, and reduced glutathione (GSH). *SOD activity* was determined spectrophotometrically [24] as a change in the absorbance at 360 nm, during autoxidation of adrenaline to adrenochrome. The enzyme activity unit (U) is the change in the absorbance by 0.001 per minute (_A/min) at 25 °C, under test conditions. *CAT activity* was determined spectrophotometrically by the Beers and Sizer method [25]. In a reaction mixture containing 1.0 mL of 0.18% aqueous H_2O_2 solution prepared by dissolving in phosphate buffer (0.05 mol/L, pH 7.0), 2 mL of phosphate buffer (0.05 mol/L, pH 7.0) and 200 L of cardiac tissue were added. A change in the absorbance due to the decomposition of H_2O_2 during the first 2 min of reaction at 260 nm was measured. The enzyme activity unit (U) is the amount of enzyme that catalyzes the degradation of 1 mol of H_2O_2 per minute, under test conditions. *GSH concentration* was determined spectrophotometrically using the glutathione recycling method. The sample was incubated in 1 mL of sodium phosphate buffer containing 0.6 mM of 5,5 = -dithio-bis-(2-nitrobenzoic acid) and 1 U/mL of GSH reductase. The reaction was initiated by adding 1.2 mM of NADPH and the change in the absorbance was monitored at 412 nm. Total GSH value was determined using the standard

curve for GSH and was expressed as nanomoles per milligram of protein.

Determination of biochemical and hematological parameters

After 15 min animals were sacrificed using a rat guillotine, and blood samples were taken. After collection, the samples were left at room temperature for 15 min and were then centrifuged (15 min × 3000 rpm) and analyzed (plasma or serum). Biochemical profile included standard biochemical parameters—glucose (GLUC), urea, creatinine (CREA), uric acid (URCA), total proteins (TP), albumins (ALB), total cholesterol (CHOL), high-density lipoproteins (HDL), low density-lipoproteins (LDL), triglycerides (TGL), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), amylase (AMY); cardiac biomarkers—high-sensitivity Troponin T (hsTnT), and lactate dehydrogenase (LDH); homocysteine metabolism markers—homocysteine, folate and vitamin B_{12}. Hematological parameters included complete blood count parameters and leukocyte subpopulations; white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), and basophils (BAS); erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT). Haemostatic parameters were measured as well (fibrinogen, von Willebrand factor and D-dimer).

Commercial kits from Siemens Healthcare Diagnostics Ltd. (Frimley, Camberley UK) and an automatic analyzer (Dimension Xpand, Siemens, Germany) was used for measurement of biochemical parameters as well Sysmex XN 1000 (Sysmex Europe GmbH, Germany) for measurement of hematological parameters. Serum homocysteine, folate and vitamin B_{12} concentrations were measured by an automated electrochemoluminescence immunoassay system ADVIA Centaur XP system (Siemens Healthcare GmbH, Erlangen, Germany). The range of reference values was Hcy < 15 µmol/L.

Immunoturbidimetric commercial assay (Siemens Healthcare GmbH, Marburg, Germany) was used for determination of fibrinogen, von Willebrand Factor (vWF), activity and antigen, D-dimer on BCS XP coagulation analyzers (Siemens Healthcare GmbH, Marburg, Germany).

Drugs

Ketamine hydrochloride 50 mg/5 mL solution and Hypnomidate (etomidate) 2 mg/mL solution was used (GlaxoSmithKline Manufacturing S.p.A. Terrile, Italy) were used in this study.

Statistical analysis

All data are presented as mean ± standard errors of mean ($X \pm$ SEM). Depending of the distribution of data, Mann–Whitney U test or Student T test was used to compare means of all tested parameters between two independent groups (K and E group). The value of p less and equal than 0.05 is considered statistically significant. Statistical analyses were performed in statistical software SPSS version 25.0 for Macintosh.

Results

The effects of etomidate and ketamine on contractile function on isolated rat heart

Left ventricular contractility (dp/dt_{max}) was significantly higher in etomidate-anesthetized rats, compared to ketamine group ($K = 669.02 \pm 33.06$ mmHg/s vs. $E = 1663.80 \pm 72.27$ mmHg/s) (Fig. 1A-a). Etomidate-anesthetized rats had better left ventricular relaxation than the ones in ketamine group (lower dp/dt_{min}) ($K = -466.58 \pm 23.96$ mmHg/s vs. $E = -1343.90 \pm 40.69$ mmHg/s) (Fig. 1A-b). There was no significant difference in the heart rate and coronary flow between these two groups (Fig. 1A-c, d).

The effects of etomidate and ketamine on redox status

Parameters of oxidative stress in coronary effluent (O_2^-, NO_2^-, H_2O_2, and TBARS) were not found to be significantly different in ketamine (K) and etomidate (E) groups (Fig. 1B-a–d).

Values of antioxidative defense parameters in cardiac tissue homogenate (SOD, CAT, and GSH) were similar in these two groups, without significant difference (Fig. 2a–c). On the other hand, activity of SOD in E group measured in haemolysate samples was significantly lower compared to the activity of this enzyme in K group (Fig. 3a). Also, GSH content was significantly changed (increased) in E group compared to the K group (Fig. 3c). Furthermore, CAT activity was not significantly different in these two groups (Fig. 3b).

The effects of etomidate and ketamine on biochemical and hematological parameters

Regarding the changes of routine biochemical parameters, we have founded that in E group levels of CREA, HDL and folate were significantly higher compared to the K group, while the levels of AMY were significantly lower in E group compared

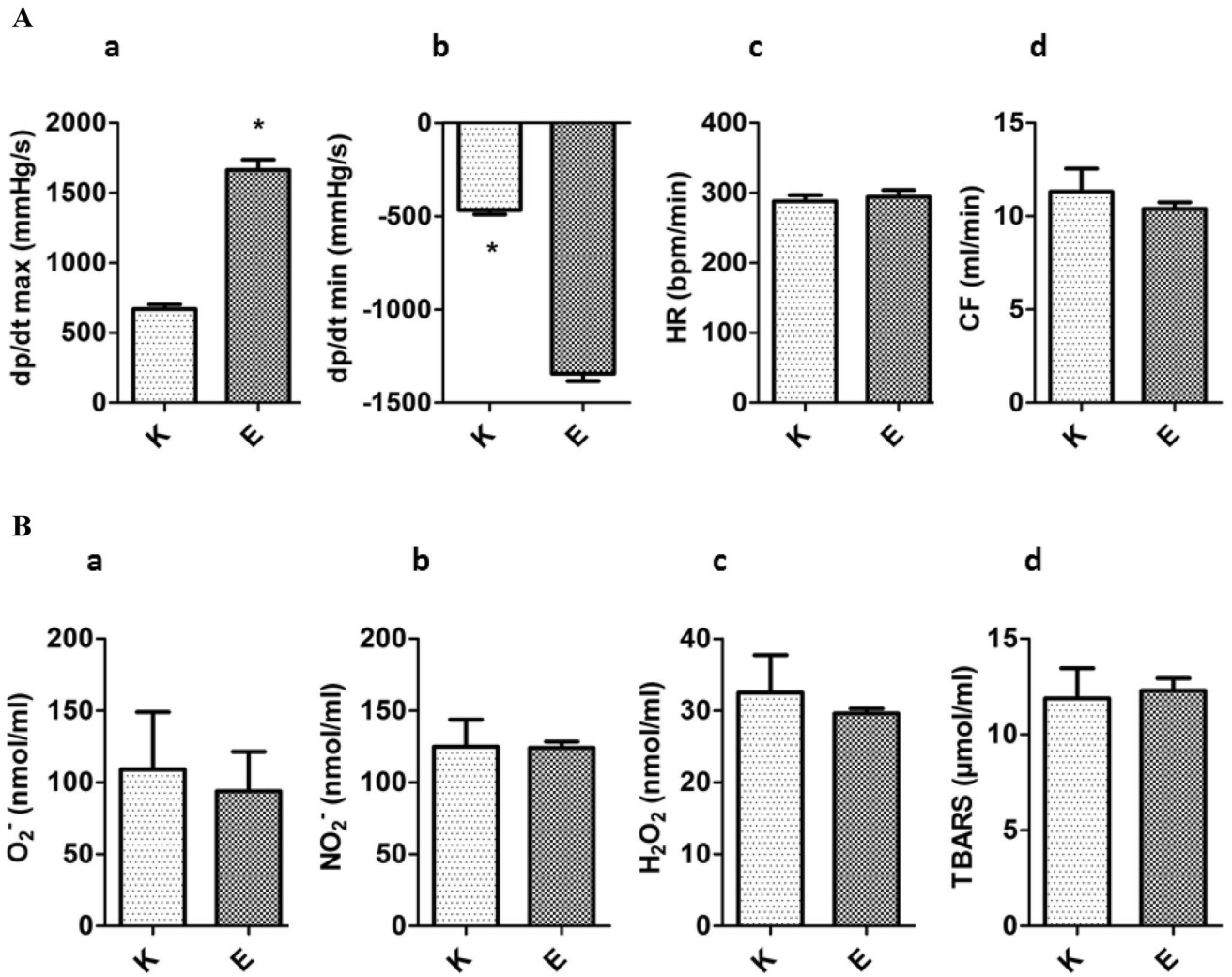

Fig. 1 **A** Cardiodynamic parameters in ketamine (*K*) or etomidate (*E*)—anaesthetized rats; a maximal rate of rise of left ventricular pressue (left ventricular contractility, dp/dt_{max}), b minimal rate of rise of left ventricular pressure (left ventricular relaxation, dp/dt_{min}), c heart rate (HR), d coronary flow (CF); Results are presented as mean ± SEM. **B** Parameters of oxidative stress in coronary effluent in ketamine (*K*) or etomidate (*E*)—anaesthetized rats; a superoxide anion radical (O_2^-), b nitrites (NO_2^-), c hydrogen peroxide (H_2O_2), d index of lipid peroxidation (TBARS), results are presented as mean ± SEM. Statistical significance is marked as asteriks (*) if p value was below or equal than 0.05

to the *K* group. Other measured parameters in serum samples were not significantly different in these two groups (Table 1).

Also, EOS concentrations in were significantly increased in ketamine-anaesthetized rats, while the hematological parameters, including haemostatic markers, were found to be similar in these two groups (Table 2).

Discussion

The present study was conducted to compare the direct effects of two anesthetic agents, ketamine and etomidate, on rat hearts and different organs by measuring levels of different biochemical and hematological parameters.

Our studies in vitro showed etomidate induced significantly increased dp/dt_{max}, but significantly decreased dp/dt_{min} compared to ketamine, which means better contractility and relaxation and depressant effect of ketamine compared to etomidate. A number of studies demonstrated negative inotropic effects of ketamine. They are presumably mediated by direct inhibition of trans-sarcolemmal calcium influx, found in guinea pig and rabbit papillary muscles [26, 27] and changes in voltage-dependent calcium channels in different animal species [28, 29]. On the other hand, there are findings demonstrating that ketamine could have positive inotropic effects. It was found in the hamster papillary muscle [29] and rat cardiac tissue as well, with low concentration of ketamine, which was almost three times higher than

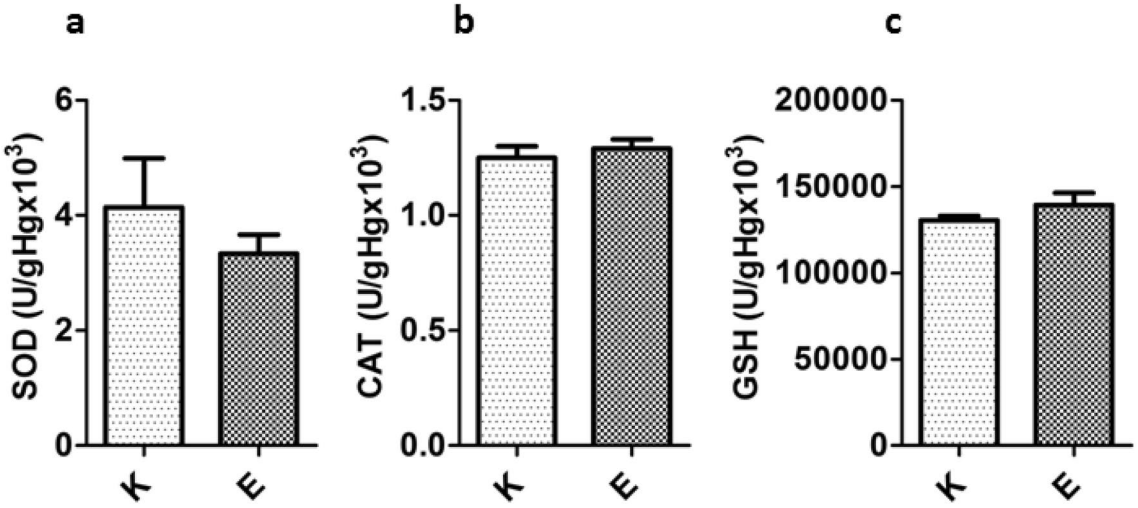

Fig. 2 Parameters of antioxidative defense in homogenate in ketamine (*K*) or etomidate (*E*)—anaesthetized rats; **a** superoxide dismutase (SOD), **b** catalase (CAT) and **c** reduced glutathione (GSH); results are presented as mean ± SEM

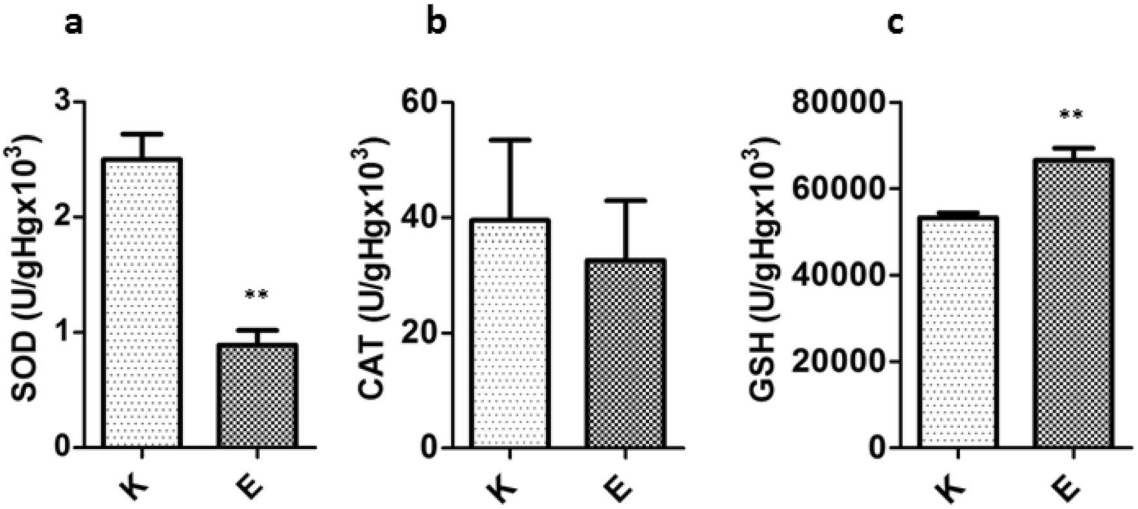

Fig. 3 Parameters of antioxidative defense in haemolysate in ketamine (*K*) or etomidate (*E*)—anaesthetized rats; **a** superoxide dismutase (SOD), **b** catalase (CAT) and **c** reduced glutathione (GSH); results are presented as mean ± SEM. Statistical significance is marked as asteriks (*) if *p* value was below or equal than 0.05, and with double (**) if was below 0.01

the one used in this study [30] Negative inotropic effects were obtained with approximately six or seven times higher concentrations then here [10, 30].

Contrary to ketamine, etomidate is shown to be devoid of negative inotropy in the heart, with minimal effects on hemodynamics and myocardial contractility [31]. Still, some authors found a decrease in left ventricular contractility and cardiac output, but only significant at supra-therapeutic concentrations [32]. Direct mechanisms, like the inhibition of the calcium current [33, 34] and decreased availability of calcium may be involved [35]. Interestingly, similar to ketamine, Riou et al. described a slight positive inotropic effect of etomidate [29]. Despite the fact that the previous studies indicate similar activities of ketamine and etomidate in the myocardium, we found significant difference in contractility and relaxation in rat heart. Etomidate induced significantly increased left ventricular contractility, but at the same time increased relaxation compared to ketamine, suggesting the impairment of sarcoplasmic reticulum function. This is corroborated by our recent study done with propofol under the same conditions [36, 37]. Compared to propofol group, ketamine provided significantly reduced contractility, and etomidate significantly increased relaxation, without changing dp/dt_{min} and dp/dt_{max}, respectively.

Table 1 Biochemical markers presented as mean ± SEM

Parameter/groups	Ketamine	Etomidate	*p* value
GLUC (mmol/L)	7.80 ± 0.62	8.93 ± 0.62	0.329
UREA (mmol/L)	8.76 ± 0.36	8.26 ± 0.21	0.180
CREA (μmol/L)	25.50 ± 2.32	39.16 ± 2.02	0.004*
URCA (μmol/L)	126.33 ± 64.64	91.00 ± 7.79	0.132
TP (g/L)	56.16 ± 2.05	57.00 ± 0.81	0.180
ALB (g/L)	31.83 ± 1.01	29.50 ± 0.42	0.065
CHOL (mmol/L)	1.37 ± 0.08	1.58 ± 0.10	0.132
HDL (mmol/L)	0.62 ± 0.03	1.40 ± 0.19	0.026*
LDL (mmol/L)	0.40 ± 0.06	0.23 ± 0.03	0.065
TGL (mmol/L)	0.76 ± 0.15	0.82 ± 0.16	0.981
AST (U/L)	181.50 ± 20.72	216.66 ± 12.68	0.180
ALT (U/L)	58.00 ± 3.74	64.33 ± 0.98	0.240
ALP (U/L)	293.83 ± 23.16	298.16 ± 18.98	0.937
AMY (U/L)	2901.33 ± 203.99	1630.66 ± 94.78	0.002*
hsTnT (ng/L)	23.33 ± 3.74	52.00 ± 16.64	0.180
LDH (U/L)	2469.66 ± 166.01	3245.33 ± 301.06	0.065
Hcy (mmol/L)	9.74 ± 1.76	8.62 ± 1.26	0.873
Fl (ng/mL)	37.71 ± 0.46	39.68 ± 0.20	0.009*
B_{12} (pg/mL)	833.00 ± 43.91	889.08 ± 32.97	0.564

Results of each parameter are compared using test for two independent variables such as Mann–Whitney test. Statistical significance is marked as asteriks (*) if *p* value was below or equal than 0.05

GLUC glucose, *UREA* urea, *CREA* creatinine, *URCA* uremic acid, *TP* total protein, *ALB* albumin, *CHOL* total cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TGL* triglycerides, *AST* aspartate amino transferase, *ALT* alanine amino transferase, *ALP* alkaline phosphatase, *AMY* amylase, *hsTnT* high-sensitivity troponin, *LDH* lactate dehydrogenase, *Hcy* homocysteine, *Fl* folate, B_{12} vitamin B_{12}

Table 2 Hematological parameters presented as mean ± SEM

Parameter/groups	Ketamine	Etomidate	*p* value
WBC (10^9/L)	10.26 ± 1.08	10.52 ± 0.80	0.892
LYM (10^9/L)	6.95 ± 0.74	7.02 ± 0.74	0.485
MONO (10^9/L)	0.61 ± 0.20	0.51 ± 0.13	0.937
NEU (10^9/L)	2.61 ± 0.39	2.86 ± 0.24	0.394
EOS (10^9/L)	0.06 ± 0.01	0.10 ± 0.01	0.041*
BAS (10^9/L)	0.01 ± 0.00	0.01 ± 0.00	0.937
RBC (10^{12}/L)	6.22 ± 0.21	6.45 ± 0.10	0.310
HGB (g/L)	121.00 ± 3.50	124.33 ± 2.56	0.699
HCT (L/L)	0.38 ± 0.01	0.39 ± 0.00	0.937
MCV (fL)	62.15 ± 0.99	60.65 ± 0.39	0.699
MCH (pg)	19.48 ± 0.28	19.26 ± 0.15	0.699
MCHC (g/L)	313.83 ± 3.55	317.83 ± 1.24	0.394
PLT (10^9/L)	880.83 ± 29.36	905.83 ± 24.17	0.589
FIB (g/L)	1.38 ± 0.12	1.25 ± 0.06	0.589
vWF (%)	133.23 ± 35.82	230.00 ± 22.00	0.132
D-dimer (mg/L)	0.24 ± 0.07	0.18 ± 0.01	0.937

Results of each parameter are compared using test for two independent variables such as Mann–Whitney test. Statistical significance is marked as asteriks (*) if *p* value was below or equal than 0.05

WBC white blood cells, *LYM* lymphocytes, *MONO* monocytes, *NEU* neutrophils, *EOS* eosinophils, *BAS* basophils, *RBC* red blood cells, *HGB* hemoglobin, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *PLT* platelets, *FIB* fibrinogen, *vWF* Von-Willebrand factor

CF was not found to be different in *K* and *E* groups. Some authors noted significant rise of CF after induction of anesthesia with ketamine [38]. However, Stowe et al. noticed a decrease in CF after ketamine, an increase after etomidate, and the biggest increase when propofol was applied in isolated guinea pig hearts [39]. Interestingly, compared to propofol group from our previous study, CF was significantly higher in both *K* and *E* groups [37]. Taking into consideration all the findings, we can say etomidate has the most favorable characteristics concerning cardiovascular system, due to increased relaxation compared to propofol and ketamine, and higher CF compared to propofol.

The current study also compares the effects of *K* and *E* on oxidative stress in haemolysate and cardiac tissue. In the literature, protective properties in traumatic and ischemia–reperfusion injuries were found in both ketamine [40, 41] and etomidate [42, 43] anesthesia. These studies demonstrate that both of them activate an antioxidative stress response, very similar. Values of antioxidative defense parameters in cardiac tissue homogenate (SOD, CAT, and GSH) were similar in these two groups, without significant difference (Fig. 2a–c). On the other hand, activity of SOD in *E* group measured in haemolysate samples was significantly lower compared to the activity of this enzyme in *K* group (Fig. 3a). Also, GSH content was significantly changed (increased) in *E* group compared to the *K* group (Fig. 3c). Furthermore, CAT activity was not significantly different in these two groups (Fig. 3b).

However, when compared with propofol from our previous study [38] significantly increased NO_2^- in coronary effluent and consequently decreased CAT in heart were found in both *K* and *E* groups. These previous results with our current results are absolutely in accordance with literature data. Actually, all general anesthetics have similar protective and antioxidant properties. But there are some little differences in comparison of each other. For example, propofol and midazolam have similar potential to diminish oxidative stress, and ketamine exhibits stronger effects by attenuating oxidative in comparison with other drugs, for example etomidate or sodium thiopental. The main way by which ketamine manifests their effect is increasing the activity of glutathione peroxidase (GPx), and superoxide dismutase (SOD). Definitely, there parameters were restored by the ketamine treatment in our study also [40, 42]. Our previous results are also similar to the effects of ketamine. Significant

decrease of SOD activity in haemolysate was observed only in *E* group in comparison with propofol group [39]. On the other hand, some studies suggested that in injured tissues was founded increased oxidative damage. So, now we know that both scenarios are possible, increased plasma level of malondialdehyde after etomidate, as well as decreased after propofol [44]. Exact antioxidative properties of *K* and *E* in various conditions are still to be explored.

Some of biochemical parameters appeared to be significantly different in these two groups. Only AMY was higher in *K* group, while CREA, folate and HDL were increased in *E* group. Some authors have found to predict anti-depressant response to monoaminergic anti-depressants by measuring the levels of folate and vitamin B_{12} [45]. As we know, ketamine is an *N*-methyl-D-aspartate (NMDA) receptor antagonist, and its single administration of at subanesthetic doses has rapid-acting anti-depressant and anxiolytic effects that occur within hours and persist for days. In our study folate levels are significantly higher in *K* group compared to the *E* group, and probably because of pharmacological properties of ketamine.

Other parameters such as CREA and AMY are also changed. Literature data confirmed this by demonstrating pronounced glucose intolerance and decreased pancreatic blood flow [46, 47]. Bostan et al. described pathological effects on the pancreas histology, but after long-term ketamine use with higher doses [14]. AMY level was also significantly higher compared with propofol from our previous experiment [39].

We found CREA to be higher during etomidate anesthesia. Nonetheless, data from literature report impaired renal function induced by ketamine abuse [48, 49]. One study did not find change of CREA levels in etomidate-infusion-anesthesia [50]. Interestingly, comparison with propofol also showed significantly increased CREA after etomidate, suggesting that etomidate could affect kidneys after single bolus administration.

Liver enzymes were not different in *K* and *E* groups. This is in line with the studies that noted increase of ALT and AST after both ketamine and etomidate application [44, 51]. Even though ketamine was reported to induce local myotoxicity and cardiotoxicity with elevation in AST and LDH [52, 53] here was not noticed the difference between these enzymes in *K* and *E* groups. It might imply that etomidate could also provide the similar effects. This is corroborated by the fact that we found significantly increased AST and LDH after etomidate compared to propofol [39].

HDL was more elevated in *E* group in comparison with *K* group. This accords with the other finding that found significantly decreased serum cholesterol and HDL levels after ketamine administration [54]. Nevertheless, other authors did not find alterations of cholesterol and HDL metabolism even after high concentrations of ketamine [55].

All the hematological parameters were mainly similar in these two groups. But, count of EOS was significantly increased in *K* group. These results suggested the immunomodulatory imbalance in rats after the ketamine administration. Data from literature showed that all of the three anesthetics—propofol, ketamine and etomidate inhibit platelet aggregation [41, 56, 57]. Moreover, Gries et al. did not find a change in PLT count after anesthesia induction with etomidate [56]. It could be assumed that propofol induced decrease of this parameter, which makes etomidate more favorable anesthetic concerning hemostatic system.

Etomidate is a unique drug used for the induction of general anesthesia and sedation. Etomidate is the only imidazole among general anesthesia inducing drugs and has the most favorable therapeutic effect following single bolus administration. It is known that the dominant molecular targets that mediate the anesthetic effects of etomidate in the central nervous system are specific γ-aminobutyric acid type A (GABAA) receptor subtypes, which had been strongly implicated in memory processes. Also, in these memory deficits are involved oxidative stress as a mechanism of action, and previous study suggested that etomidate could decrease oxidative stress by increasing of SOD and GSH.

Furthermore, anesthetics, especially etomidate and ketamine could contribute in adrenal dysfunction in critically patients, for example in patients with sepsis. As we know, mechanisms of adrenal dysfunction, particularly in course of sepsis or septic shock, may include impaired availability of cleaving of the substrate (HDL)-cholesterol and steroidogenic enzymes. In this course, some types of leukocytes also could be impaired, as a result of inflammation process [45].

Taken in consideration everything discussed, we can deem that etomidate provides more favorable effects in comparison to ketamine, due to increased ventricular contractility and relaxation and higher HDL. Etomidate has also more beneficial effect compared to propofol regarding cardiovascular system, due to increased relaxation and CF. In overall performance, propofol still remains the most appropriate anesthetic, since etomidate showed elevated CREA, AST, LDH and oxidative stress.

One limitation to our study is lack of a control group, as all animals received either ketamine or etomidate prior to being sacrificed. Even though we cannot determine the extent of changes induced by these two components compared with a no-drug group, we avoided to perform it on not anesthetized animals, as it is probably not in accordance with ethical guidelines. Other experiments in the literature which compare different anesthetics also lack a real control group [58–60].

Conclusion

It could be concluded that etomidate has more beneficial effects on myocardial contractility but ketamine has higher antioxidant potential compared to etomidate. The present study gives a good insight into general effects of these anesthetics. This is important for their clinical use and the right choice of the anesthetic agent in a specific case, considering their effects on different organs. It can also be helpful for the development of analogues of etomidate that will have the most desirable safety profile regarding to the cardiovascular function.

Acknowledgements This work was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia, Grant Number 175043.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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