# Propofol-Induced Mitochondrial and Contractile Dysfunction of the Rat Ventricular Myocardium

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## Summary

Propofol is a short-acting hypnotic agent used in human medicine for sedation and general anesthesia. Its administration can be associated with serious cardiovascular side-effects that include decrease in arterial blood pressure and cardiac output. The aim of the present study was to evaluate propofol effects on mitochondrial respiration, myocardial contractility and electrophysiology in the same samples isolated from the heart ventricles of adult rats. Mitochondrial oxygen consumption was measured in permeabilized samples dissected from free walls of both ventricles using high-resolution respirometry. State LEAK was determined with malate and glutamate. Active respiration was induced by ADP (state PI) and further by succinate, a Complex II substrate (PI+II). Rotenone was injected to measure state PII. Antimycin A, a Complex III inhibitor was used to determine residual oxygen consumption (ROX). N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride and ascorbate were injected simultaneously for respirometric assay of cytochrome c oxidase activity (CIV). Isometric contractions and membrane potentials were determined on multicellular preparations isolated from right and left ventricles. Propofol concentrations used ranged from 0.005 to 0.5 mmol/l. All respiratory parameters were significantly higher in the left control ventricles compared to the right ones. Propofol significantly decreased Complex I activity at concentration 0.025 mmol/l and papillary muscle contraction force at 0.1 mmol/l. Propofol did not affect action potential duration at any concentration studied. Our study suggests that mechanisms contributing to the impaired myocardial contraction during propofol anesthesia might include also mitochondrial dysfunction manifested by compromised activity of the respiratory Complex I.

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## Introduction

Propofol (2,6-diisopropylphenol) is a short-acting hypnotic agent used in human medicine for sedation and general anesthesia (Bryson *et al.* 1995). Its administration can be associated with serious cardiovascular side-effects that include decrease in arterial blood pressure and cardiac output (Yang *et al.* 2015). In rare cases, propofol causes life-threatening propofol infusion syndrome characterized by bradycardia, severe arrhythmias, rhabdomyolysis of the myocardium and skeletal muscles, heart failure, renal insufficiency, hepatomegaly and metabolic acidosis (Krajčová *et al.* 2015).

Many direct effects of propofol on cardiomyocytes potentially causing dose-dependent decrease in cardiac contractility have been suggested to

date. They include calcium-related events on both sarcolemmal and subcellular levels: decrease in ICaL amplitude (Liu *et al.* 2008), modulation of Na-Ca exchange activity (Wickley *et al.* 2007), decrease in sarcoplasmic reticulum calcium load (Sprung *et al.* 2001) or impaired sensitivity of myofilaments to calcium ions (Wickley *et al.* 2006). In contrast, data documenting no significant impact of propofol on cardiac contractility have been also repeatedly reported (Riou *et al.* 1992, van Klarenbosch *et al.* 2001). Concerning the influence of propofol on mitochondria, both harmful and beneficial effects have been recorded to date. Recently, Vanlander *et al.* (2015) have suggested possible mechanism of propofol infusion syndrome *via* impeding the electron flow through respiratory chain and coenzyme Q. On the other hand, potential protective effects of propofol on ischemia-induced myocardial injury mediated by mitochondrial ATP-sensitive potassium channels, inhibition of the mitochondrial permeability transition (Sztark *et al.* 1995) or limitation of reactive oxygen species production in mitochondria have been documented (Shao *et al.* 2008). As these contradictory results could arise from various experimental setups, where animals of different species, strains and ages were used in addition to wide range of propofol concentrations in a variety of vehicle solutions, we have decided to test effects of propofol on membrane electrophysiology, force of contraction and mitochondrial oxygen consumption in multicellular preparations isolated from both ventricles of male adult rats.

## Methods

### Animals and experimental design

A total of 37 Wistar 6-month-old male rats (VELAZ, Prague, Czech Republic) were used for all experiments conducted in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU) and the relevant Guidelines of the Czech Ministry of Agriculture for Scientific Experimentation on Animals and approved by the University Committee for Experiments on Laboratory Animals. We used exclusively mature adult male rats to reduce the number of animals required to perform all planned experiments and to gain results comparable to available experimental data. At the day of experiment, animals were killed by cervical dislocation and their hearts were quickly excised. For electrophysiological measurements, the papillary

muscles were dissected from both left and right ventricles. For high-resolution respirometry, small samples (approx. 20 mg of tissue) were taken from the subendocardial layer of anterior free walls of both ventricles. If not stated otherwise, all chemicals were of analytical grade and were purchased from Sigma-Aldrich (Prague, Czech Republic).

### High resolution respirometry

Samples of ventricular tissues were placed into ice-cold preservation BIOPS solution of the following composition (in mmol/l): $CaK_2EGTA$ 2.77, $K_2EGTA$ 7.23, $Na_2ATP$ 5.77, $MgCl_2 \cdot 6H_2O$ 6.56, taurine 20, $Na_2$Phospho-creatine 15, imidazole 20, dithiothreitol 0.5 and MES hydrate 50 at pH 7.1 and 0 °C (Kuznetsov *et al.* 2004) and gently mechanically permeabilized with a pair of sharp forceps to get a specimen approximately doubled in volume and pale in color (Pesta and Gnaiger 2012).

Then the fibers were added into BIOPS containing saponin (50 µg/ml) for 10 min and washed twice with respiration medium with catalase (MiR06) for 10 min. MiR06 contains (in mmol/l): EGTA 0.5, $MgCl_2 \cdot 6H_2O$ 3, K-lactobionate 60, taurine 20, $KH_2PO_4$ 10, Hepes 20, sucrose 110, and fatty acid free bovine serum albumin 1 g/l at pH 7.1 and 37 °C (Pesta and Gnaiger 2012). The fibers were then blotted dry, weighed, and used for high-resolution respirometry.

Oxygen consumption by cardiac muscle fibers was measured at 37 °C in 2-ml glass chambers of oxygraph Oroboros (Oroboros, Innsbruck, Austria) connected to the computer with DatLab software for data acquisition and analysis (Oroboros, Innsbruck, Austria). All oxygen fluxes were corrected for instrumental and chemical background measured in separate experiments performed in the same medium without biological samples.

After equilibration (40 min with air), oxygen concentration in the chamber corresponded to its concentration in the atmospheric air and solubility in the medium (0.92). Then, permeabilized fibers were added into the medium, the chambers were closed and oxygen concentration was raised to 400-500 mmol/l by titration of $H_2O_2$ (200 mmol/l). After stabilization, combination of substrates and inhibitors was sequentially injected into the chambers to measure the respiration through different segments of the electron transport system (SUIT protocol; Fig. 1): 1) Resting respiration with substrates providing electrons to Complex I malate (2 mmol/l) and glutamate (10 mmol/l) was measured as a state S2 (non-

phosphorylating LEAK state, L). 2) Active respiration was induced by 5 mmol/l adenosine diphosphate (ADP; state S3 or OXPHOS; PI). 3) Integrity of the mitochondrial inner membrane was checked with cytochrome c (10 µmol/l). 4) Mitochondrial respiration was then increased by succinate, a Complex II substrate (10 mmol/l; PI+II). 5) After addition of a Complex I inhibitor rotenone, the oxygen flux corresponded to maximum capacity of the electron-transporting system with the Complex II only (PII). 6) Then, antimycin A (2.5 µmol/l), a Complex III inhibitor was injected into the chambers to measure residual oxygen consumption (ROX).        7) N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD; 0.5 mmol/l) and ascorbate (2 mmol/l) were injected simultaneously for respirometric assay of cytochrome c oxidase activity (CIV).

Propofol was dissolved in absolute ethanol and injected into the chambers 20 min prior to the SUIT protocol in concentrations ranging from 0.005 mmol/l to 0.5 mmol/l. The volume of ethanol injected was 1 µl per chamber. The effect of ethanol (up to 5 µl) on oxygen flux was measured in separate experiments; no changes in respirometric parameters were detected.
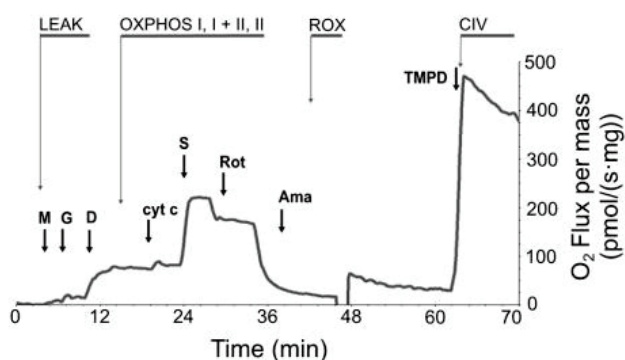


**Fig. 1.** Substrate-uncoupler-inhibitor titration (SUIT) protocol with substrates for Complex I, Complex II, and Complex IV in the rat heart. Black line – oxygen flux expressed per mg tissue wet weight. M – malate, G – glutamate, D – ADP, c – cytochrome c, S – succinate, Rot – rotenone, AmA – antimycin A, TMPD – N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride and ascorbate.

*Citrate synthase activity*

As citrate synthase activity may serve as a marker of mitochondrial content in the analyzed tissues and cells, we determined its activity in the samples aspirated from each oxygraph chamber (Kuznetsov *et al.* 2002, Larsen *et al.* 2012). The assay medium (800 µl; 0.1 mmol/l 5,5-dithio-bis-(2-nitrobenzoic) acid, 0.25 % Triton-X, 0.5 mmol/l oxalacetate, 0.31 mmol/l acetyl coenzyme A,

5 µmo/l EDTA, 5 mmol/l triethanolamine hydrochloride, and 0.1 mol/l Tris-HCl, pH 8.1) was mixed with 200 µl of the homogenized chamber content. The enzyme activity was measured spectrophotometrically at 412 nm and 30 °C over 200 s and expressed in IU per mg tissue weight (Kuznetsov *et al.* 2002).

*Electrophysiology and contractility measurements*

Papillary muscles or trabeculae were dissected from the right or the left ventricle and placed into an experimental chamber perfused with oxygenated Tyrode solution (36 °C) at a constant flow rate (6-10 ml/min). Tyrode solution contained (in mmol/l): NaCl 137; KCl 4.5; $MgCl_2$ 1; $CaCl_2$ 2; glucose 10; HEPES 5; and pH was adjusted to 7.4 with NaOH. After stabilization period (30 min), the steady-state contraction force and membrane potential were measured using an isometric force transducer (model F30; Hugo Sachs Electronik – Harvard Apparatus, GmBH, March-Hugstetten, Germany) and glass microelectrodes filled with 3M KCl (resistance >20 MΩ; Microelectrode Puller P-1000, Sutter Instrument, CA, USA). Measurements were performed at stimulation frequencies 1, 2, 3, and 5 Hz (Pulsemaster Multi-Channel Stimulator A300; World Precision Instruments, Inc., Sarasota, FL, USA). After the signals in control solution had been obtained, propofol was added sequentially to the Tyrode solution to reach following concentrations: 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mmol/l. Signals were recorded using the National Instruments data-acquisition hardware and software (National Instruments Corporation, Austin, TX, USA).

MATLAB 2014b (MathWorks Inc., Natick, MA, USA, 2014) was applied for offline signal analysis. The action potential duration (APD) was measured at the 50 % and 90 % levels of repolarization (APD50, APD90). Time course of the contraction force was described by three parameters: time-to-peak (TTP), time-to-50 %-relaxation (R50), and time-to-90 %-relaxation (R90). The resting tension was taken as zero and then maximal amplitude of the contraction force (CFmax) was determined. All parameters were measured in at least ten beats, averaged, and the mean values were used for further analyses and comparisons.

*Data analysis and statistics*

Results are presented as mean ± SD or SEM. Statistical differences were analyzed using software package STATISTICA Cz, version 8 (StatSoft Inc., 2007,

Prague, Czech Republic) or OriginPro 8.5 (OriginLab Corporation, Northampton, MA, USA, 2011). After testing for the normality of distribution and homogeneity of variances, comparisons were made using one-way ANOVA (with *post-hoc* LSD or Bonferroni test) or Student's t-test, where appropriate. The results were considered significantly different when p<0.05. Fifty per cent effective concentration was calculated using OriginPro 8.5 from the dose-response curve fitting (with 95 % confidence limits).

## Results

### High resolution respirometry

As shown in Figure 2, respiratory parameters PI, PI+II, PII, and CIV were significantly higher in the left ventricles than in the right ones. In the left ventricle, propofol significantly decreased oxygen consumption by Complex I (PI) at concentration 0.025 mmol/l and its effect was even more expressed at higher concentrations of the drug. In the right ventricle, propofol concentration significantly compromising mitochondrial ADP-stimulated respiration by Complex I (PI) was 0.05 mmol/l. The effect of propofol on PI was concentration dependent; the EC50s calculated from the fitted dose-response curve were 0.023±0.05 and 0.049±0.04 mmol/l for the left and right ventricle, respectively. In contrast, propofol did not have any substantial effect on Complex II or Complex IV related active respiration at concentrations lower than 0.5 mmol/l (Fig. 3A-D). Although the drug did not significantly impact the absolute values of the LEAK state, the OXPHOS control ratio L/P increased with increasing propofol concentration suggesting lower efficiency of the phosphorylating process. In the left ventricle, control L/P ratio was 0.19±0.01 and it significantly increased from propofol concentration 0.05 mmol/l (L/P=0.25±0.07) to 0.5 mmol/l (L/P=0.69±0.14). In the right ventricle, the OXPHOS control ratio in the intact samples was 0.21±0.02 and reached significantly higher value at 0.025 mmol/l propofol concentration (L/P=0.28±0.04) and further increased with rising drug level.

Citrate synthase activity was not affected by propofol at any concentration studied.

### Electrophysiology and contractility measurements

In both heart ventricles there was no significant effect of propofol on the action potential duration (APD) at any of propofol concentrations, regardless of the stimulation frequency (1, 2, 3, or 5 Hz, Fig. 4A). Kinetics of the contraction-relaxation cycle were not influenced by propofol except the highest concentration tested (0.5 mmol/l), at which the times to 50 % relaxation (R50) and 90 % relaxation (R90) were significantly prolonged in both left and right ventricles (Fig. 4B for stimulation frequency 1 Hz). The time to peak of contraction force (TTP) remained unchanged at all propofol concentrations.

The application of propofol reduced contraction force at all stimulation frequencies used (1, 2, 3, and 5 Hz, Fig. 5A-D). In the right ventricle, a significant decrease was observed at lower concentrations than in the left ventricle. However, the analysis of dose-response curves did not reveal any major difference in EC50 at any stimulation frequency: 0.33±0.02 (1 Hz), 0.24±0.03 (2 Hz), 0.21±0.04 (3 Hz), 0.31±0.05 (5 Hz) mmol/l in the left ventricle and 0.39±0.03 (1 Hz), 0.23±0.03 (2 Hz), 0.22±0.03 (3 Hz), and 0.20±0.03 (5 Hz) mmol/l in the right ventricle.
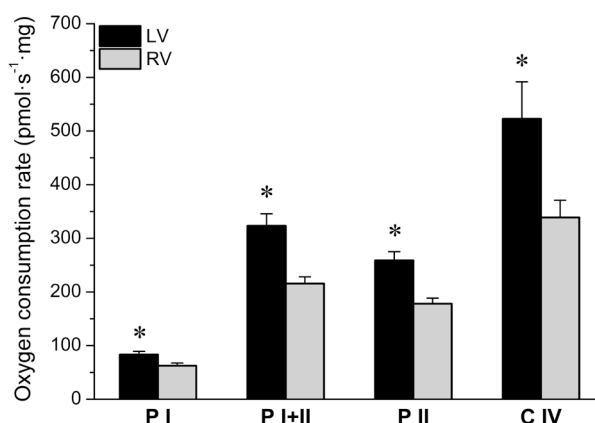


**Fig. 2.** Oxygen consumption rates in the state OXPHOS and Complex IV activity measured under control conditions in the right and left ventricles (RV, LV, respectively). PI – oxygen consumption rate in the state OXPHOS in the presence of ADP and substrates providing electrons to Complex I, i.e. glutamate and malate; PI+II – OXPHOS in the presence of ADP, glutamate, malate and a Complex II substrate, succinate; PII – OXPHOS in the presence of ADP and succinate, Complex I blocked by rotenone; CIV – Complex IV oxygen consumption rate in the presence of ascorbate and TMPD, corrected for autooxidation. Oxygen fluxes were expressed per mg tissue wet weight. * P<0.05, LV compared to RV.

## Discussion

The results of the present study indicate that propofol, in clinically relevant concentrations, substantially affects mitochondrial respiration and thus phosphorylation capacity in the mammalian heart. Compromised energy supply can contribute to the well-known effects of propofol on the cardiac contractility.
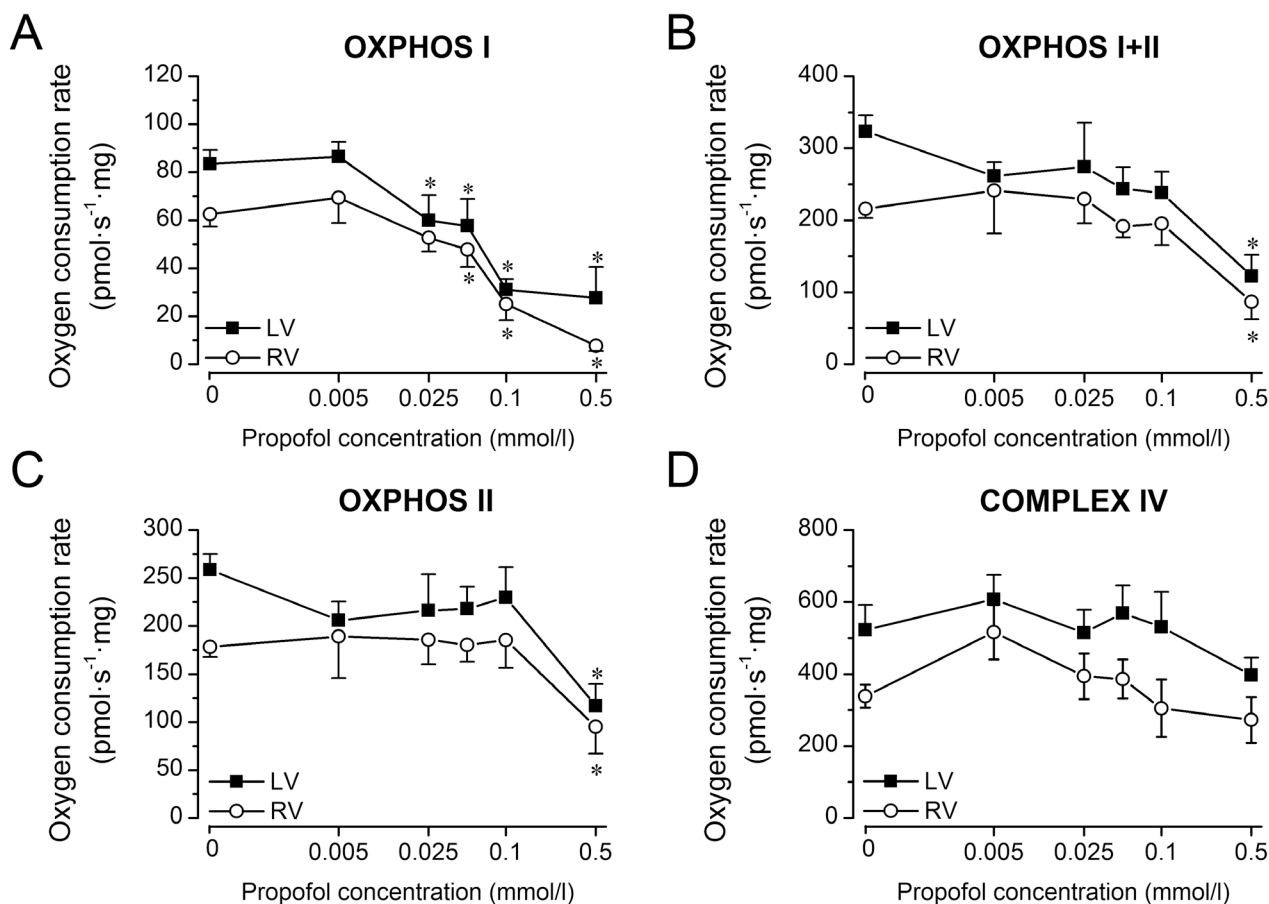
**Fig. 3.** Respiration of the permeabilized fibers isolated from the left (LV) and right ventricles (RV; cca 1 mg/ml). Oxygen consumption was measured in MiR06 medium as described in Materials and Methods. Propofol effect was tested in concentrations 0.005, 0.025, 0.05, 0.1, and 0.5 mmol/l. **A.** Respiration after malate (2 mmol/l), glutamate (10 mmol/l), and ADP (5 mmol/l; PI). **B.** Oxygen consumption after malate, glutamate, ADP, and succinate (10 mmol/l; PI+II). **C.** Active respiration after ADP, succinate rotenone (0.5 µmol/l; PII). **D.** Oxygen consumption after antimycin A (2.5 µmol/l), ascorbate (2 mmol/l), and TMPD (0.5 mmol/l; Complex IV). * P<0.05, significantly different from the respective control.
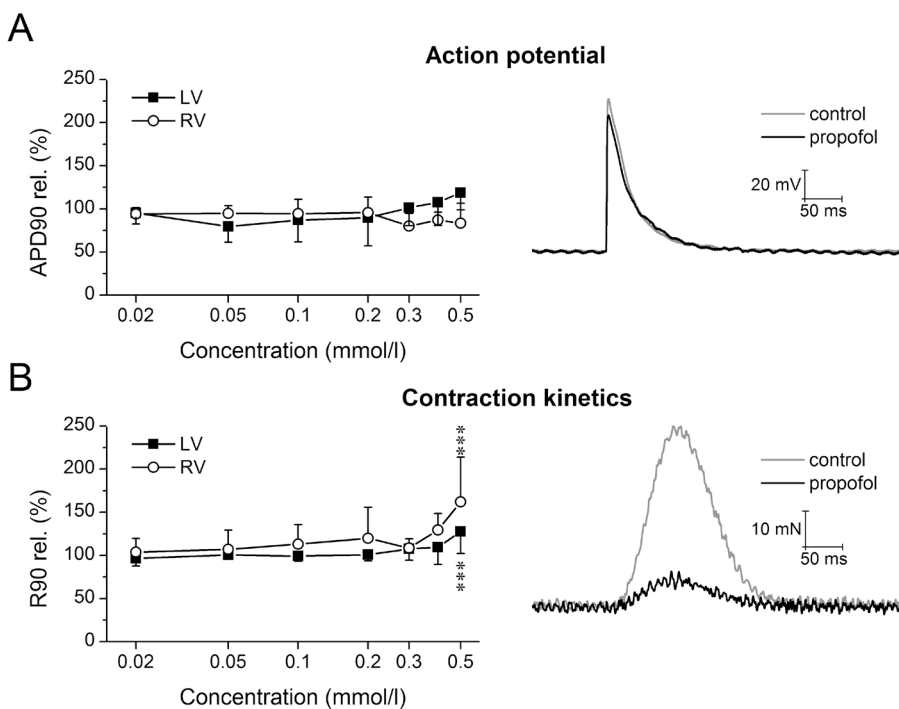


**Fig. 4.** Effect of propofol on the time course of action potential and contraction force. **A.** Action potential duration at 90 % level of repolarization in the left (LV) and right (RV) ventricle at stimulation frequency of 1 Hz at various propofol concentrations expressed as a percentage of values obtained in control solution. Right panel – representative membrane potential traces in solution with (0.5 mmol/l) and without propofol. **B.** Time-to-90 %-relaxation repolarization in left (LV) and right (RV) ventricle at stimulation frequency 1 Hz at various propofol concentrations expressed as a percentage of values obtained in control solution. Right panel – representative contraction traces in solution with (0.5 mmol/l) and without propofol. *** P<0.001, significantly different from the control.
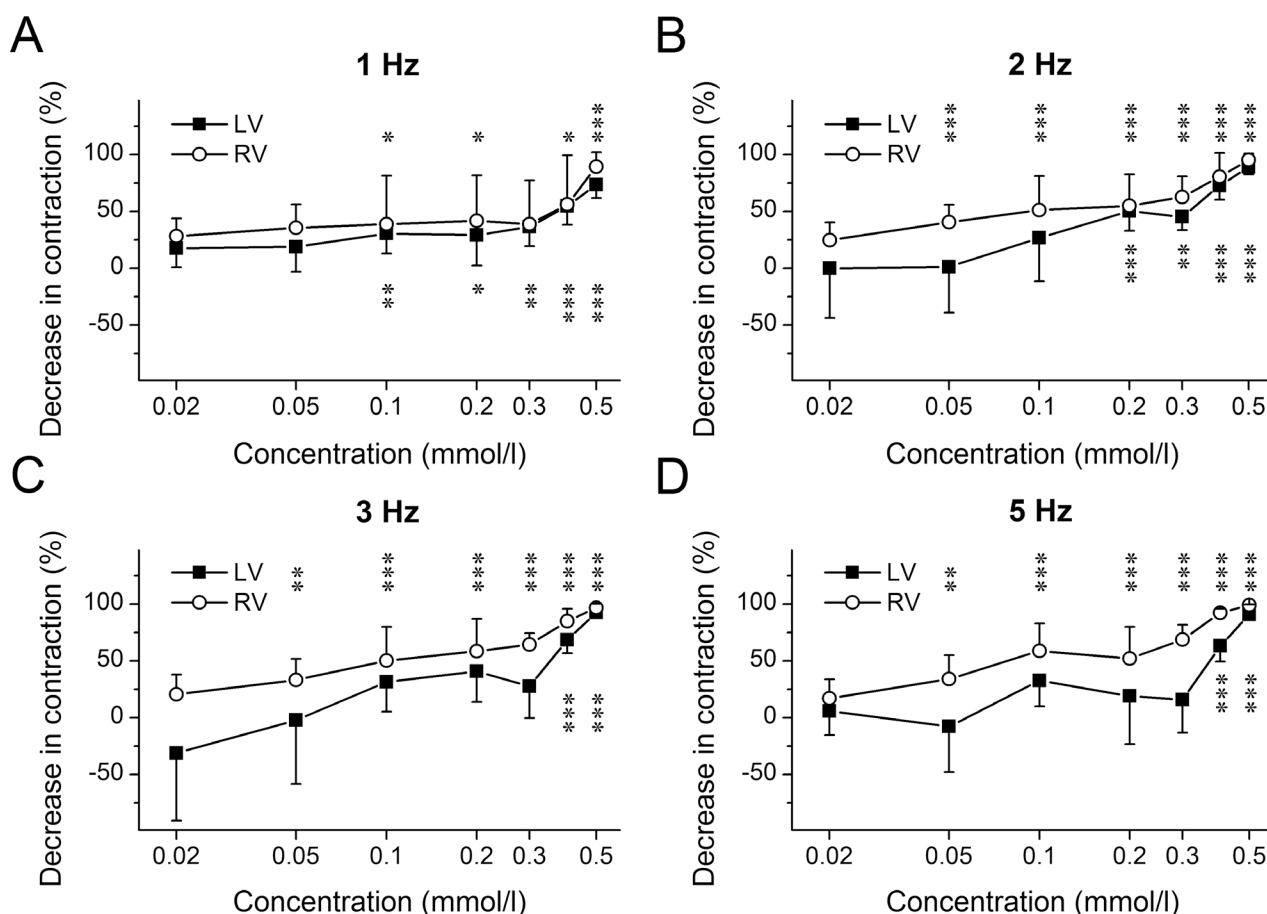
**Fig. 5.** Concentration dependence of the negative inotropic effect of propofol at different stimulation frequencies. Decrease in contraction in the left (LV; full squares) and right (RV; open circles) ventricle at stimulation frequencies 1 (**A**), 2 (**B**), 3 (**C**), and 5 Hz (**D**) expressed as a percentage of values obtained in control solution. * P<0.05, ** P<0.01, *** P<0.001, significantly different from the control.

Plasma concentrations of propofol determined in anesthetized patients range from 5 to 50 µmol/l, those above 30 µmol/l are frequently considered high (Soehle *et al.* 2015). It should be noted that in human plasma, propofol is extensively bound to albumin and red blood cells and its free fraction represents only 1 to 5 % of the total concentration, i.e. 0.05 to 2.5 µmol/l (Mazoit and Samii 1999, Dawidowicz *et al.* 2008). The range of concentrations used in our experiments was selected according to known propofol levels in humans and rats and with regard to concentrations reported to cause significant changes in cardiac functions (Grounds *et al.* 1985). The lowest concentration used in our oxygraphic experiments, i.e. 5 µmol/l was still higher than that of free propofol calculated from higher total drug level. Nevertheless, we still consider our concentrations clinically relevant for several reasons: 1) During induction of general anesthesia, propofol free concentration can exceed several fold the steady state values (Bienert *et al.* 2012). 2) Free fraction of propofol can be increased by various drugs that could bind to the same site on albumin, conformational alterations of molecular albumin or changes in plasma protein concentration that also limit propofol binding to albumin (Dawidowicz *et al.* 2008). 3) Solution MiR06 used in our experiments contained 1 g/l of albumin (fatty acid free, globulin free) that could bind substantial portion of propofol added into the oxygraph chambers. One gram of albumin per liter represents approx. 15 µmol of binding sites for propofol that is normally bound to the drug site I in close proximity to one of fatty acid binding sites with high affinity (Simard *et al.* 2006).

A limited number of experimental studies addressed the potential impact of propofol on mitochondrial respiration in the heart, although drug-induced depression of cardiac function is known and feared side-effect of propofol. In the isolated rat heart mitochondria, propofol decreased both the

transmembrane potential and ATP synthesis, however at concentrations by far exceeding those reported for free drug fraction during general anesthesia (Branca *et al.* 1995). Shao *et al.* (2008) reported no effect of propofol at concentration 100 µmol/l on the activities of the respiratory complexes in mitochondria isolated from the rat heart. In the same concentration, propofol protected mitochondrial function and decreased cardiac injury by prevention of cardiolipin peroxidation during ischemia and reperfusion (Shao *et al.* 2008). In our study, we used mitochondria *in situ*, i.e. in permeabilized muscle fibers, where the total population of mitochondria with preserved intracellular interactions is accessible for determination of oxygen consumption (Doerrier *et al.* 2016).

Our study has not revealed any change in action potential duration at any of the propofol concentrations used. The data concerning the effect of propofol on electrophysiological parameters of the rat heart are scarce. A single study documents no change in duration of action potential of the rat right ventricular papillary muscle up to concentration of propofol 300 µmol/l (Azuma *et al.* 1993). However, studies on isolated cardiomyocytes demonstrated significant inhibition of ICaL at propofol concentrations 100 µmol/l (Liu *et al.* 2008) and even 25 µmol/l (Zhou *et al.* 1997). Moreover, there are also data documenting some impact of propofol on potassium currents in the rat ventricular cardiomyocytes. Delayed rectifier $K^+$ current was decreased at 36 µmol/l of propofol in the clonal cardiac cells H9c2 established from embryonic ventricle (Liu *et al.* 2008), but not at concentration approaching clinical values, when measured in rat isolated ventricular cardiomyocytes (2.5 µmol/l; Carnes *et al.* 1997). Our results are not in conflict with these findings, since decreases in calcium and potassium membrane currents have opposite effects on action potential duration (Lee *et al.* 2016) and may therefore cancel each other.

The present study shows that contractility of the rat ventricular myocardium is significantly inhibited at propofol concentration 50 µmol/l. The 50 % effective concentrations at the most relevant stimulation frequency 3 Hz were 210 and 230 µmol/l in the left and right ventricles, respectively. Studies on the effect of propofol on the force of myocardial contraction are relatively numerous, but extremely controversial in relation to the mammalian species or experimental approach used (multicellular preparations, isolated cardiomyocytes). In the rat heart papillary muscle, no contraction-compromising effect of propofol was observed up to

600 µmol/l (Azuma *et al.* 1993). However, their results were obtained under different experimental conditions, i.e. low temperature (30 °C) and stimulation frequency irrelevant for the rat heart (0.5 Hz). Majority of studies on isolated cardiomyocytes of different species including rat report EC50 for decrease in force of contraction of ~50 µmol/l (Hamilton *et al.* 2000, Puttick and Terrar 1992). The decline in the force of contraction could not be fully explained by a decrease in L-type calcium current and was suggested to be contributed by other processes dealing with calcium turnover in the cell, like calcium sensitivity, modulation of Na-Ca exchange activity, decrease in sarcoplasmic reticulum calcium load or impaired sensitivity of myofilaments to calcium ions (Hamilton *et al.* 2000, Wickley *et al.* 2007, Sprung *et al.* 2001, Wickley *et al.* 2006).

*Study limitations*

In the present study, the lowest concentration of propofol having significant effect on the heart function was 25 µmol/l, which seems to be at least 10 times higher than is the free propofol concentration in the human plasma. However, proven accumulation of propofol in tissues, substantially higher plasma concentrations of the drug during induction of general anesthesia, duration of infusion, various comorbidities and unknown genetic background of mitochondrial dysfunction could lead to the heart dysfunction induced by even lower plasma concentrations of propofol (Bienert *et al.* 2012, Krajčová *et al.* 2015, Vanlander *et al.* 2015).

This study addressed neither putative gender-related differences in the propofol sensitivity (Maeda *et al.* 2016), nor precise molecular mechanism of the drug effect at Complex I level or link between mitochondrial function and heart contraction.

## Conclusion

Our study suggests that mechanism contributing to the impaired myocardial contraction during propofol anesthesia might include also mitochondrial dysfunction manifested by compromised activity of the respiratory Complex I. Our findings support hypothesis of potential critical involvement of mitochondrial dysfunction in the pathophysiology of the propofol infusion syndrome (Finsterer and Segall 2010).

## Conflict of Interest
There is no conflict of interest.

## Acknowledgements

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