Possible Pathogenic Mechanism of Propofol Infusion Syndrome Involves Coenzyme Q

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ABSTRACT

Background: Propofol is a short-acting intravenous anesthetic agent. In rare conditions, a life-threatening complication known as propofol infusion syndrome can occur. The pathophysiologic mechanism is still unknown. Some studies suggested that propofol acts as uncoupling agent, others suggested that it inhibits complex I or complex IV, or causes increased oxidation of cytochrome c and cytochrome aa_3, or inhibits mitochondrial fatty acid metabolism. Although the exact site of interaction is not known, most hypotheses point to the direction of the mitochondria.

Methods: Eight rats were ventilated and sedated with propofol up to 20 h. Sequential biopsy specimens were taken from liver and skeletal muscle and used for determination of respiratory chain activities and propofol concentration. Activities were also measured in skeletal muscle from a patient who died of propofol infusion syndrome.

Results: In rats, authors detected a decrease in complex II+III activity starting at low tissue concentration of propofol (20 to 25 µM), further declining at higher concentrations. Before starting anesthesia, the complex II+III/citrate synthase activity ratio in liver was 0.46 (0.25) and in skeletal muscle 0.23 (0.05) (mean [SD]). After 20 h of anesthesia, the ratios declined to 0.17 (0.03) and 0.12 (0.02), respectively. When measured individually, the activities of complexes II and III remained normal. Skeletal muscle from one patient taken in the acute phase of propofol infusion syndrome also shows a selective decrease in complex II+III activity (z-score: –2.96).

Conclusion: Propofol impedes the electron flow through the respiratory chain and coenzyme Q is the main site of interaction with propofol. (**ANESTHESIOLOGY 2015; 122:343-52**)

P**ROPOFOL** is administered for induction and maintenance of anesthesia and for sedation.[1] Since 1990, a syndrome associated with propofol sedation has been recognized, which received the name propofol infusion syndrome (PRIS).[2] Bray *et al.*[3] formulated criteria for PRIS: occurrence of bradycardia combined with one of the following conditions: (1) clinically enlarged liver, or fatty infiltration at autopsy, (2) lipemic plasma, (3) metabolic acidosis with base excess less than –10 mM, or (4) signs of skeletal muscle involvement assessed by myoglobinuria or rhabdomyolysis. In addition, lactic acidosis, arrhythmia, renal, cardiac, and circulatory failure can occur. Most victims were sedated for long time (>48 h) and with high doses (>4 mg kg^{-1} h^{-1}).

Multiple studies indicate that propofol has an effect on the respiratory chain. A decrease in mitochondrial

What We Already Know about This Topic

- Propofol infusion syndrome is an uncommon life-threatening complication of propofol administration observed most often in patients receiving high doses for a long time
- The underlying pathophysiologic mechanism of propofol infusion syndrome is thought to involve direct interference with mitochondrial energy production

What This Article Tells Us That Is New

- In eight rats sedated for up to 20 h with gradually increasing doses of propofol, succinate cytochrome c reductase (complex II+III) was the most sensitive to inhibition by propofol
- The activities of complex II and complex III were not decreased when tested individually, suggesting that propofol interferes with coenzyme Q, which transfers electrons from complex II to complex III

transmembrane electrical potential ($\Delta\Psi$) was detected in liver mitochondria isolated from control rats incubated with propofol at concentrations between 0 and 75 µM. The rate of oxygen consumption was increased suggesting that propofol acts as an uncoupler.[4] Also, in rat synaptosomes, propofol was shown to decrease $\Delta\Psi$ at concentrations between 30 and 100 µM[5] Incubating mitochondria from rats using high concentrations of propofol (100 to 400 µM) resulted in a strong inhibition of the activity of complex I, and to a lesser degree of complex II+III.[6] In one report, a reduction of complex IV activity was found in skeletal muscle, which led to the hypothesis that a propofol metabolite caused disruption of the respiratory chain.[7] Perfusing guinea pig heart muscle with propofol resulted in an increase in myoglobin oxygen saturation and increased oxidation of cytochrome c and cytochrome aa$_3$.[8] These findings argue against the hypothesis that propofol acts as uncoupler. Kam *et al*.[9] hypothesized that the inhibitory action of propofol is caused by inhibition of coenzyme Q at complex II, of cytochrome c at complex IV, or by inhibition of carnitine palmitoyltransferase I and II, but did not test this hypothesis through experimentation. Prolonged infusion of propofol in rabbits induced fatal multiorgan dysfunction similar to PRIS in humans.[10] Another study in rabbits showed adenosine monophosphate-activated protein kinase activation in heart muscle, a cellular response to lowered adenosine triphosphate levels.[11] In an 18-month-old child who developed PRIS shortly after infusion of propofol (5 h), lowered complex IV activity was detected in skeletal muscle.[12] Complex IV deficiency was also found in a 10-month-old boy who survived PRIS after treatment by hemodialysis.[7] Other reports suggested possible interaction of propofol with fatty acid oxidation.[13–15] Taken together, these findings suggest a failure in energy production as the causative mechanism, implying mitochondria, more specifically the respiratory chain, as the most interesting pathway for the study of the pathophysiologic mechanism of PRIS.

To gain better insight into the interaction of propofol with the respiratory chain, we have sedated and mechanically ventilated rats for several hours using gradually increasing doses of propofol. At several time points, blood and tissue samples were taken which were used for biochemical analyses and measurement of propofol concentrations and its metabolites. In another experiment, skeletal muscle homogenates from control rats were incubated with propofol.

Materials and Methods

Animals and Instrumentation

The study protocol was approved by the Ethics Committee (approval 08/04EDC) for Animals of Ghent University Hospital (Ethische Commissie Dierproeven, faculteit Geneeskunde en Gezondheidswetenschappen, UGent, Ghent, Belgium). Male Wistar rats (240 to 360 g) were kept at 21°C with a 12 h to 12 h light–dark cycle. *Ad libitum* access to food and water was provided. The catheters were

placed after administration of ketamine (120 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Animals were instrumented for invasive blood pressure, heart rate, and rectal body temperature recording. Polyethylene (PE) catheters were inserted into the femoral (PE 10) and carotid artery and into the external jugular and femoral vein (PE 50). Arterial blood pressure, central venous pressure, and cardiac rhythm were monitored on a Sirecust 1281 monitor (Siemens, Munich, Germany). The core temperature was maintained with a feedback system using a flexible probe inserted rectally to a depth of 2 cm. Mechanical ventilation was performed with a Servo 300 ventilator (Siemens) through a tracheal cannula (internal diameter 2.0 mm). Ventilatory conditions were initially set at peak inspiratory pressure, 20 cm H_2O; positive end-expiratory pressure, 3 cm H_2O; tidal volume, 10 to 12 ml/kg; and frequency, 70 to 90 min^{-1} and regulated in function of blood gas analysis. Fluid and glucose demand was supplied by glucose 5% infusion (5 to 10 mg kg^{-1} h^{-1}). Analgesia was achieved by remifentanil. The initial dose (50 µg kg^{-1} min^{-1}) was further titrated on a clinical parameter (pedal withdrawal reflex). Whole experimental procedure was completed for eight rats. Experiments with three rats were aborted prematurely due to the development of acute metabolic acidosis and ventricular tachyarrhythmia (See Supplemental Digital Content 1, http://links.lww.com/ALN/B105, a figure representing a rhythm strip of a propofol treated rat).

Experimental Protocol for Animal Studies

After instrumentation, rats were sedated with propofol (Diprivan®; AstraZeneca, Macclesfield, Chesire, United Kingdom) initially at a dose of 20 mg kg^{-1} h^{-1}. At the start of the experiment, blood gas analysis was performed on a mobile device (I-stat®; Abbott, Princeton, NJ). Biochemical tests in serum were used to monitor the possible occurrence of PRIS (potassium, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, creatinine, lactate, and acylcarnitine). The same measurements were repeated after 6 and 12 h. Analysis of the acylcarnitine profile was performed by tandem mass spectrophotometric analysis using a protocol by Zytkovicz *et al*.[16]

Biopsies were taken from skeletal muscle (m. gastrocnemius, left hind leg) and liver (left lobe) at 6 and 12 h after the start of the sedation by propofol (T1 and T2, respectively). Depending on the duration of the total sedation–ventilation procedure, T3 was set between 3.5 and 8 h after T2. The ventricular heart muscle biopsy was taken at the end of the sedation–ventilation protocol (at T3). Rats were sedated with gradually increasing doses of propofol. At T1, sedation had been performed for 6 h at 20 mg kg^{-1} h^{-1}, at T2 for 6 extra hours at 40 mg kg^{-1} h^{-1}, and at T3 for a period ranging between 3.5 and 8 h at 80 mg kg^{-1} h^{-1}. Samples were immediately stored on ice water.

Control samples were obtained from skeletal muscle (m. gastrocnemius, left hind leg), liver (left lobe), and

ventricular heart tissue from male Wistar rats which had been allocated to other experiments and sacrificed by cervical dislocation. Consequently, rats could not be randomized to control and treatment groups. The control samples were taken before any drug was administered. Rat anesthesia, tissue sampling, and subsequent spectrophotometric measurements were performed by the same researcher excluding the possibility of sample blinding.

Preparation of Tissue Homogenates

Samples were immediately stored at –80°C. Mitochondrial enriched fractions from skeletal muscle, liver, and heart muscle were prepared as described previously by Scholte et al.[17] Briefly, skeletal muscle (25 to 66 mg), liver (22 to 67 mg), and heart muscle (47 to 63 mg) were homogenized in a glass/glass pestle in a tissue to buffer ratio of 1:20 (10 mM Tris-HCl, 0.25 M sucrose, 2.0 mM EDTA, 50 U/ml heparin, and pH 7.4). The homogenates were centrifuged (5,600g) for 1 min. The supernatant was kept on ice and sonicated prior to spectrophotometric analysis. All homogenates were prepared on the same day using the same stock solutions.

Spectrophotometric Analyses of Respiratory Chain Function and Krebs Cycle Enzymes

The activities of the respiratory chain complexes were measured in an ultraviolet/visible spectrophotometer (Ultrospec 2100 pro®; Amersham Bioscience, Corston, Bath, United Kingdom). Nicotinamide adenine dinucleotide:coenzyme Q reductase (complex I) was measured according to Fischer et al.,[18] succinate reductase according to Rustin et al.,[19] succinate coenzyme Q reductase (complex II) according to Hatefi and Stiggall,[20] succinate cytochrome c reductase (complex II+III activity) according to Sottocasa et al.,[21] cytochrome c ubiquinol reductase (complex III) according to Birch-Machin et al.,[22] cytochrome c oxidase (complex IV) according to DiMauro et al.,[23] and citrate synthase (CS) according to the protocol of Srere.[24] To limit variability, all spectrophotometric analyses were performed using the same stock solutions prepared at the beginning of the experiments, except for oxaloacetic acid that was freshly prepared for every experimental series. Enzyme activities of malate dehydrogenase, fumarase, and pyruvate dehydrogenase were measured as described previously.[25–27]

Spectrophotometric analyses were performed in homogenates prepared from biopsies collected at different time points (T1, T2, and T3). In addition to the respiratory chain complexes, CS activity was measured. CS is an enzyme located in the mitochondrial matrix encoded by nuclear DNA and its activity reflects the mitochondrial content. Expressing the respiratory chain activity as the ratio over CS normalizes the activity for mitochondrial content and allows comparison between different samples.[28] During each run of enzyme activity testing, a standard was used to evaluate interrun variability (See Supplemental Digital Content 2, http://links.lww.com/ALN/B106, a figure representing a box-and-whisker plot showing interrun variability of activity ratios for each complex assay). This standard was prepared from hearts of male Wistar rat. The observed variability meets previously reported values.[29]

In Vitro Test of the Inhibitory Effects of Propofol and Corresponding Derivatives

Either propofol (2,6-diisopropylphenol, SAFC supply solutions; Sigma-Aldrich Chemie, Steinheim, Germany), the oxidized form of propofol (2,6-diisopropyl-1-4-quinone, in-house synthesis) or reduced form of propofol (2,6 diisopropyl-1,4-quinol, in-house synthesis) was added to the reaction mixture (skeletal muscle homogenates from control rats) at increasing concentrations (100, 200, and 400 μM). After 3 min of incubation, the reaction was started according to the above-mentioned protocols. As propofol was dissolved in ethanol, an equal amount of ethanol (without propofol) was used for preincubation in the control assays. For experiments evaluating the possible rescue effect of coenzyme Q on the activity of complex II+III, coenzyme Q (final concentrations 0.13 and 0.26 mM) was added to the mitochondrial sample after 3 min of incubation with propofol. After 1 extra minute of incubation with coenzyme Q, the assay was started.

Protein Determination

Protein concentrations in the samples taken in vivo were assayed by the Pierce Coomassie Plus Protein Assay Kit (Product No. 23236) (Thermo Fisher Scientific, Rockford, IL) as described by Bradford.[30] For the in vitro samples, the protein content was assayed by the modified Lowry Protein assay kit (Product No. 23240) (Thermo Fisher Scientific) based on the method originally described by Lowry et al.[31]

Blue Native Polyacrylamide Gel Electrophoresis and High-resolution Clear Native Polyacrylamide Gel Electrophoresis

Blue native polyacrylamide gel electrophoresis (PAGE) with in-gel activity staining was performed as described by Van Coster et al.[32] and Smet et al.[33] High-resolution clear native-PAGE was performed according to the methodology described by Wittig et al.[34]

Propofol Determination in Tissue Homogenates

Determination of propofol and its quinol and quinone derivatives (2,6-diisopropyl-1-4-quinone and 2,6 diisopropyl-1,4-quinol) in human skeletal muscle and in animal tissue homogenates (skeletal muscle, liver, and heart muscle) was performed according to a modified protocol of Court et al.[35] using a reversed phase separation and subsequent ultraviolet and fluorescence detection. In brief, 500 μl tissue homogenate (approx. 0.5 mg total protein) was added to 200 μl saturated NaCl solution in a Teflon-sealed glass vessel under gentle mixing. In addition, 100 μl of Lipofundin® MCT [medium chain triglycerides] 20% (B. Braun, Melsungen AG, Germany) was added as solubilizer and 20 μl thymol dissolved in ethanol

(88 µM) as internal standard. Samples were extracted twice with 2.0 ml diethyl ether, vigorously mixed for 10 min using a vibramax mixer (1,800 rpm), and centrifuged at 1,500*g* max for 5 min at 5°C. Organic layers were collected in a glass tube and then dried down at 50°C under a stream of nitrogen. Residues were dissolved in 250 µl dimethyl sulfoxide acidified with 1.0 mM HCl. For injection into the high-performance liquid chromatography system, 10 µl of the dissolved samples was used. The high-performance liquid chromatography system consisted of a System Gold® (JASCO; Groß-Umstandt, Germany) Analog Interface Module 406, a System Gold® (JASCO) Solvent Module 127 as a pump connected to a Jasco 851-AS autosampler (JASCO). A reversed-phase column (LiChrospher-100, 250 mm × 4.6 mm × 5 µm; Merck, Darmstadt, Germany) was used for chromatography. Temperature of the column was maintained at 23°C. The high-performance liquid chromatography mobile phase consisted of 50% acetonitrile, 40% H_2O, and 10% methanol. A flow rate of 400 µl/min was applied. Detection of propofol and its quinol derivative was done by using Shimadzu RF-551 as a fluorescence detector (λ_{ex} = 276 nm, λ_{em} = 310 nm), whereas for analysis of the quinone derivative of propofol, a System Gold® Scanning Detector Module 167 (JASCO) as an ultraviolet detector (λ = 270 nm) was used.

Statistical Analysis

Statistical analysis was performed with statistical software IBM, SPSS Statistics 19® (Armonk, NY). For comparing two groups, the nonparametric Mann–Whitney U test was used. For comparing more than two groups, the nonparametric Kruskal–Wallis test for unpaired values was used and one-way ANOVA for comparing means of paired values with *post hoc* Bonferroni correction. For evaluation of the effect of coenzyme Q on the interaction between propofol and complex II+III, we performed a regression model on the log-transformed values, testing the significance of the interaction terms, *i.e.*, propofol concentration, enzyme activity, and coenzyme Q concentration. The interaction term reflects the differences in the slopes across the different plots. A *P* value of less than 0.05 was considered significant (*P < 0.05, **P < 0.01, and ***P < 0.001). Data were plotted in scatter plots or box-and-whisker plots. Sample size calculation could not be performed due to the limited amount of information available on the *in vivo* effects of propofol on rats. The reason to opt for eight rats in the experimental condition and four rats in the control condition was based on technical considerations. Indeed, using this number of rats offered the advantage that all samples could be analyzed in the same batch which allowed to minimize variations between measurements.

Results

In Vivo *Animal Study*
Propofol and Propofol Derivative Concentrations. The concentration of propofol and its metabolites was measured in tissue samples taken from rats treated with propofol at different time points (fig. 1). In liver, the concentrations of propofol were 40, 80, and 180 µM at T1, T2, and T3, respectively (fig. 1A). In skeletal muscle, the concentrations of propofol were lower, ranging from 10 to 20 µM, and 50 µM at T1, T2, and T3, respectively (fig. 1B). Propofol concentration was the least elevated in heart muscle, reaching 25 µM at T3 (fig. 1C). None of the oxidized (quinone) or reduced (quinol) derivatives could be detected in any of the tissue samples.

Catalytic Activities of Respiratory Chain Complexes. Succinate cytochrome c reductase (complex II+III) was the enzyme that was the most sensitive to inhibition by propofol. In liver, complex II+III showed a significant decline in activity at an average tissue concentration of 40 µM of propofol measured at T1 (fig. 2A). In skeletal muscle, a decline in the activity of complex II+III was noticed at an average tissue concentration of 20 µM of propofol (at T2) (fig. 2B) and in heart muscle at an average tissue concentration of propofol of 25 µM (at T3) (fig. 2C). The activities of the individual complexes II and III, on the other hand, were not affected (data not shown), not even in the samples with the highest concentration of propofol (180 µM). The enzyme complex that was the second most sensitive to inhibition by propofol was complex IV. In liver, the activity of complex IV was significantly decreased at 40 µM of propofol (at T1) (fig. 2D) and in skeletal muscle at 50 µM (at T3) (fig. 2E). The activity of complex I showed no decline in any tissue at any time point (data not shown).

In-gel Activity Staining after Separation of the Respiratory Chain Complexes by Blue Native-PAGE and High-resolution Clear Native-PAGE. After separation of the respiratory chain complexes by blue native-PAGE, in-gel activity staining was performed for complex II and V. After separation of the complexes by high-resolution clear native-PAGE, in-gel activity staining was performed for complexes I and IV. Using these staining methods, catalytic activity of the individual complexes I, II, IV, and V was found unaltered
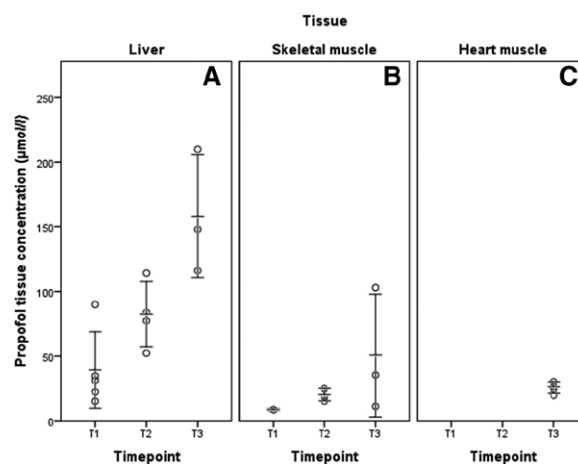

Fig. 1. Tissue concentrations of propofol are shown at different time points (T1, T2, and T3) in liver (*A*), skeletal muscle (*B*), and at T3 for heart muscle (*C*). Individual measurements are indicated by *open circles*. *Whiskers* indicate mean ± SD.
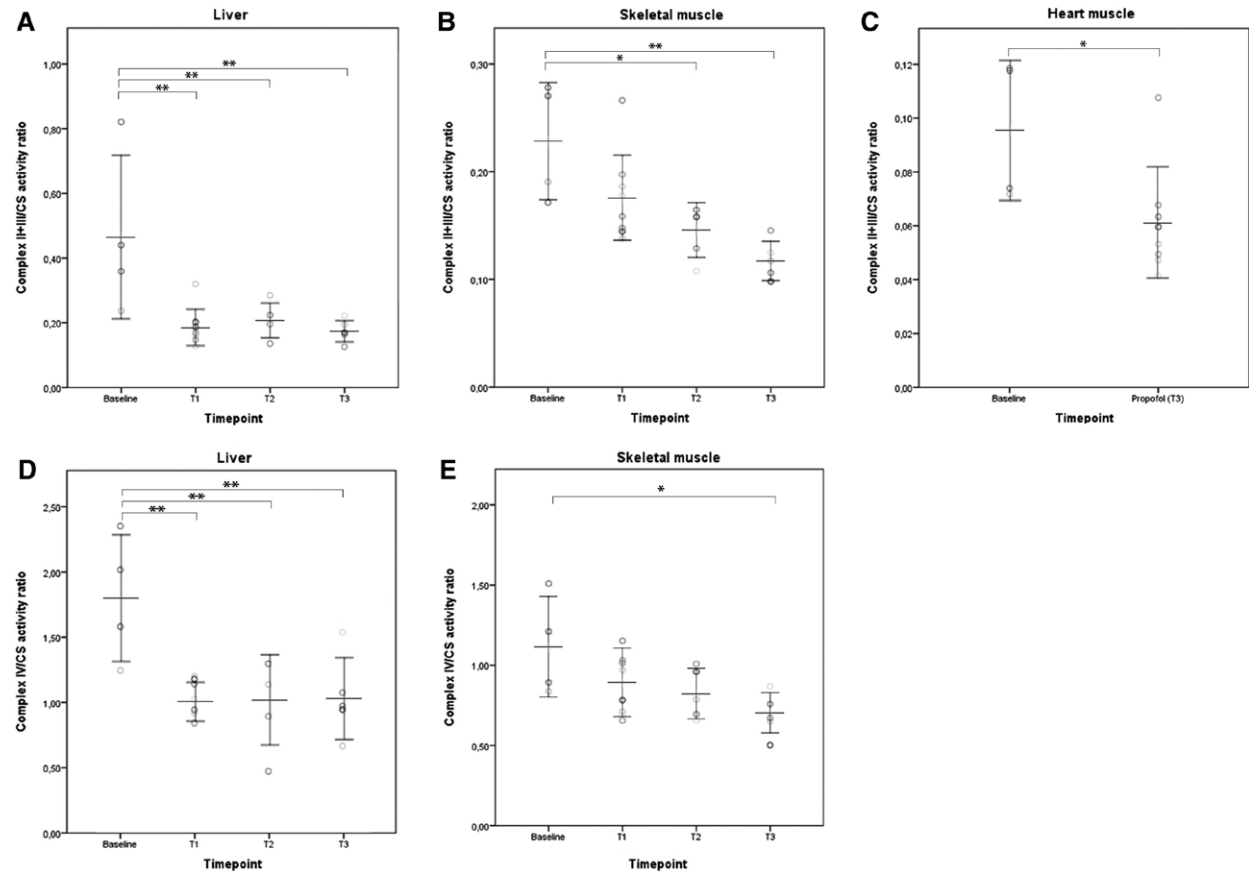
Fig. 2. Scatter plots for the activity ratios for complex II+III over citrate synthase (CS) at several time points (T1, T2, and T3) compared with baseline in liver (*A*), skeletal muscle (*B*), and heart muscle (*C*) and the activity ratio for complex IV over CS at different time points in liver (*D*) and skeletal muscle (*E*). Individual measurements are indicated by *open circles*. *Whiskers* indicate mean ± SD. Significance between different time points is indicated by a *horizontal bracket*. *$P < 0.05$ and **$P < 0.01$.

for all tissues tested (fig. 3). Complex III activity could not be visualized by catalytic staining in the high-resolution clear native-PAGE gel, neither in controls nor in propofol-treated

Fig. 3. In-gel activity staining for complex II and V after blue native polyacrylamide gel electrophoresis separation and for complex I and IV after high-resolution clear native polyacrylamide gel electrophoresis separation in different tissues from control rats and from propofol (P)-treated rats. The protein band of complex III is only visible in the marker lane.

samples. Coomassie staining showed a normal amount of protein in complex III (data not shown).

Blood Tests. The metabolic condition of the animals during the anesthetic procedure was assessed by blood gas sampling at T0, T1, and T2. As seen in table 1, blood gases remained in the normal range for each measurement, indicating stable metabolic and ventilatory conditions during the whole procedure. To detect early biochemical signs of PRIS, blood sampling was performed at T0, T1, and T2. In two rats, a significant increase of creatine kinase, aspartate aminotransferase, alanine aminotransferase, and lactate (rats 3 and 4) was seen. Both rats developed ventricular tachyarrhythmia. In the other rats, significant increases in these parameters in serum were not observed. At the same time points, blood was also collected on a Guthrie card to assess the acylcarnitine profile. In these blood samples, no alteration could be detected (data not shown), in particular an increase in C2- or C5-acylcarnitine, as earlier reported in PRIS victims,[13,14] could not be observed.

To show that the respiratory chain complexes were selectively targeted by propofol, the effect of propofol was tested on several Krebs cycle enzymes such as malate dehydrogenase, fumarase, and CS, and on pyruvate dehydrogenase
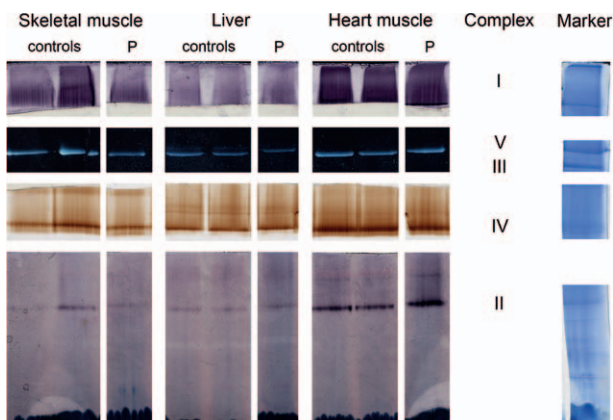
Table 1. Arterial Blood Gas Analysis from Anesthetized Rats

	pH			HCO$_3^-$ (mmol/l)			pCO$_2$ (mmHg)		
	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3
Baseline (n = 8)	7.32	7.37	7.39	24.3	25.7	27.5	41.2	45.2	50.1
T1 (n = 4)	7.22	7.31	7.35	14.3	20.2	24.8	33.2	39.4	46.7
T2 (n = 4)	7.22	7.28	7.34	20.2	21.0	23.9	45.0	46.4	48.8

Arterial blood gas analyses (pH, HCO$_3^-$, and pCO$_2$) for the anesthetized rats at different time points (T1: 6 h after starting the procedure; T2: 12 h after starting the procedure). Values represent Q1 (25th percentile)—median—Q3 (75th percentile).
HCO$_3^-$ = bicarbonate concentration in arterial blood; n = number of rats; pCO$_2$ = partial carbon dioxide pressure in arterial blood.

complex. A significant effect on these enzymes could not be demonstrated (data not shown).

An effect of the propofol derivatives (quinol or quinone) could not be evaluated using the spectrophotometric assays as both products were highly reactive and distorted the measurements, even without adding any tissue sample.

In Vitro *Propofol Incubation Experiments*

Skeletal muscle homogenates from untreated rats were tested after preincubation with propofol at three different concentrations for 3 min at 37°C. Final concentrations of propofol in the reaction mixture were 100, 200, and 400 µM. Remarkably, preincubation with propofol at 100 µM did not result in a significant decrease in the activities of complex II+III (fig. 4A) and complex IV (fig. 4B). Also complex I (fig. 4C), complex II (succinate dehydrogenase, see fig. 4D; succinate coenzyme Q reductase, see fig. 4E), and complex III (fig. 4F)

were not inhibited at this concentration. This is in contrast with the *in vivo* experiments where it was shown that in skeletal muscle, a propofol concentration at 20 µM already caused a significant reduction of activity, especially of complex II+III. Only at a 10-fold higher concentration of propofol (200 µM), an inhibitory effect on the respiratory chain activities was seen *in vitro*. Under these conditions, the activities of several respiratory chain complexes were decreased, including complex I (fig. 4C), complex II+III (fig. 4A), complex III (fig. 4F), and complex IV (fig. 4B). An inhibition of the activity of complex II (fig. 4, D and E) was seen at a preincubation concentration of propofol at 400 µM.

To confirm the results of the *in vivo* experiments showing a decrease in the complex II+III activity in the presence of propofol, an *in vitro* experiment was set up by adding extra coenzyme Q to the homogenate after the incubation with propofol. Coenzyme Q at either a final concentration of 0.13 or 0.26 mM was
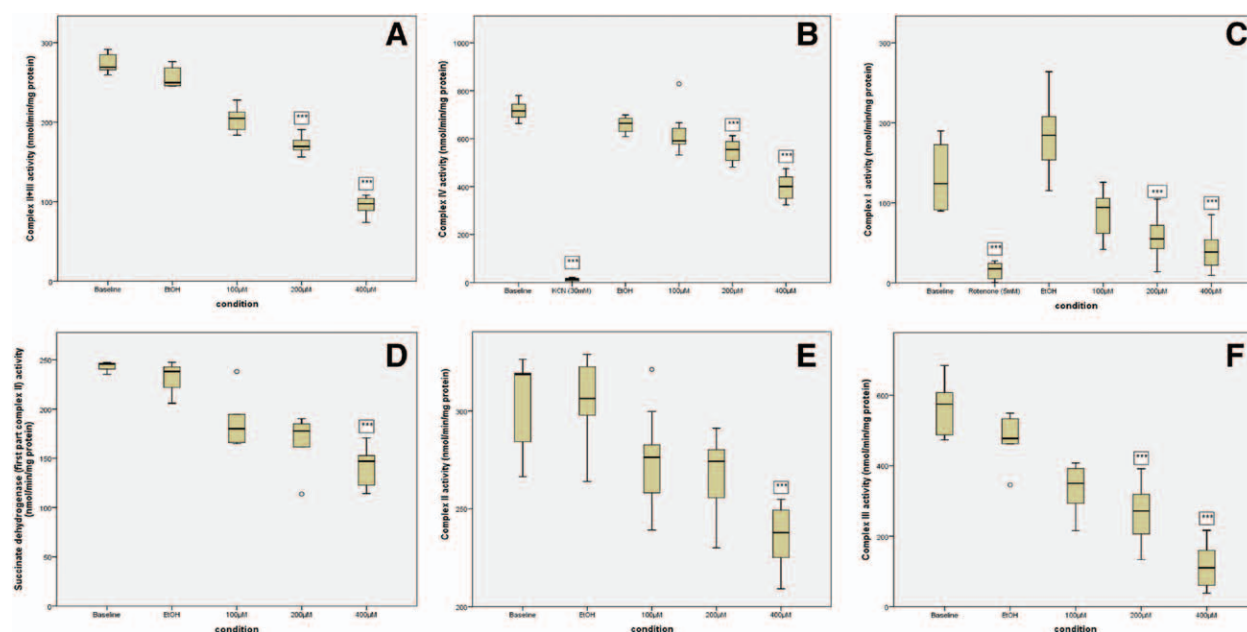

Fig. 4. Box-and-whisker plots showing the effect of increasing doses of propofol and the vector molecule (EtOH, ethanol) on the enzymatic activity (expressed as nmol min^{-1} mg^{-1} protein) in skeletal muscle homogenates of complex II+III (*A*), complex IV (*B*), complex I (*C*), succinate dehydrogenase (*D*), complex II (*E*), and complex III (*F*). Significant inhibition of the activity is seen after preincubation at 200 and 400 µM of propofol for complex II+III, IV, I and III and at 400 µM for succinate dehydrogenase and complex II. Outliers are indicated by a small *open circle*. Significance is expressed against "baseline" condition. ***$P < 0.001$.
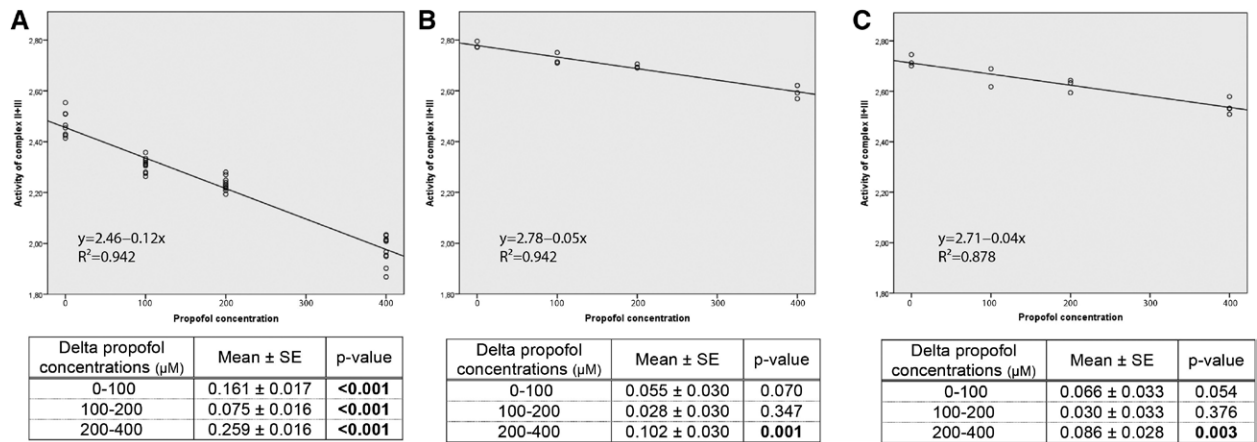
Fig. 5. Scatter plots with line of best fit expressing the activities of complex II+III at increasing propofol concentrations (expressed in µM) and at different coenzyme Q concentrations: no additional coenzyme Q added (*A*), 0.13 mM coenzyme Q added (*B*), and 0.26 mM coenzyme Q added (*C*). The *insets* represent the curve equation and the coefficient of determination, R^2. The significance of the interaction term between the propofol concentration and the coenzyme Q concentration is less than 0.001. The tables below the plots represent the mean difference (mean ± standard error) of complex II+III enzyme activity between the successive increasing propofol concentrations, for the respective coenzyme Q concentrations. The *P* value represents the significance of the effect of propofol on the enzyme activity of complex II+III.

able to cause a significantly smaller decrease in enzyme activity of complex II+III after propofol incubation (fig. 5).

Propofol Concentration and Respiratory Chain Activities in Postmortem Skeletal Muscle from a Human PRIS Victim

A 39-yr-old Caucasian male with a medical antecedent of diabetes mellitus type 1 was admitted to an intensive care unit after being involved in a road traffic accident resulting in severe brain injury. Sedation with propofol was started shortly after arrival. His mean infusion dose was 2.96 mg kg^{-1} h^{-1}, with a maximum dose of 3.0 mg kg^{-1} h^{-1}, for a total infusion time of 115 h. He contracted septic shock with high vasopressor need and he progressed toward renal failure. On the fifth day of admission, he suffered from acute arrhythmia due to hyperkalemia (7.6 meq/l, normal, 3.5 to 5.1) which was likely to be caused by a combination of rhabdomyolysis (creatine kinase up to 17,671 U/l; normal, 57 to 374) and renal failure. Continuous veno-venous hemofiltration was started but did not result in an improvement of his condition. PRIS was diagnosed based on the clinical presentation

Fig. 6. In-gel activity for all oxidative phosphorylation complexes extracted from skeletal muscle after separation by blue native polyacrylamide gel electrophoresis from a propofol infusion syndrome victim (P) showing no impaired activities as compared with control (C).

and prolonged administration of propofol. The patient died shortly thereafter. Spectrophotometric analysis in a postmortem skeletal muscle biopsy showed a significantly decreased activity of complex II+III (*z*-score: –2.96) and of complex IV (*z*-score: –3.66), and only mild impairment of complex I (*z*-score: –1.59) and complex III (*z*-score: –1.75). The propofol concentration in skeletal muscle was 38 µg propofol per gram tissue, corresponding to 10 µM. Separation of the respiratory chain complexes by blue native-PAGE followed by in-gel activity staining revealed normal complex activities (fig. 6).

Discussion

Although propofol is considered a safe agent for sedation and anesthesia, a severe complication can occur especially in the patients receiving high doses for a longer period. From the literature, it is not clear what the underlying pathophysiologic mechanism is, although the majority of observations point into the direction of interference with the energy production in the mitochondria.[4–6,8] In this study, based on the results of an *in vivo* study in animals and biochemical studies in postmortem tissues from a patient affected by PRIS, more insight was gained in how prolonged administration of propofol can lead to failure of energy production in the mitochondria.

From the results of the *in vivo* experiment in rats, it became clear that propofol accumulated faster in liver than in skeletal muscle. Also, the concentrations in liver reached much higher values than in skeletal muscle. The concentration of propofol in heart muscle remained relatively low. Succinate cytochrome c reductase (complex II+III) was the enzyme that was the most sensitive to inhibition by propofol. A significant decline in its activity was already observed at relatively low concentrations of propofol (20 µM in skeletal muscle). In contrast, when
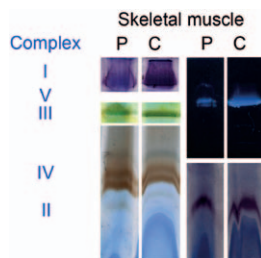
tested individually, the activities of complex II and complex III were not decreased at this concentration of propofol. This can only be explained by interference of propofol with coenzyme Q. The latter is a lipophilic molecule embedded in the inner mitochondrial membrane that transfers electrons from complex II to complex III and from complex I to complex III. In our *in vivo* experiments, we detected that at higher tissue concentrations of propofol (40 µM in liver and 50 µM in skeletal muscle), complex IV also started to show a slight decline in activity which can be explained by the fact that propofol also competes with the cytochrome aa_3 in complex IV at this concentration.

The structure of propofol resembles that of the catalytic part of coenzyme Q (fig. 7). The latter is a quinone derivative with a long isoprenoid tail that renders the molecule highly nonpolar and enables it to diffuse rapidly into the hydrocarbon phase of the inner mitochondrial membrane.[36] Propofol is an even smaller molecule with high lipophilic character resulting in higher membrane solubility.[37] Propofol is also known to be a potent antioxidant.[38] It can take over the role of ubiquinone by accepting electrons from complex I and II but without transferring these to complex III, thereby interrupting the electron flow. For complex I, a vast number of different substances had inhibitory effects on the catalytic activity because these derivatives compete with coenzyme Q at its binding domain.[39–41] The inhibitory effect of propofol on complex IV activity cannot be linked to interaction with coenzyme Q but to a direct redox interaction between propofol and reduced cytochrome c, or between propofol and cytochrome aa_3. As shown earlier by Schenkman and Yan,[8] perfusion of heart muscle with propofol resulted in more oxidation of cytochrome c and cytochrome aa_3 (in complex IV) and delayed their reduction in ischemic conditions.

In the second part of this study, the activities of the complexes of the respiratory chain were measured in skeletal muscle homogenates from control rats after preincubation *in vitro* with propofol (100 to 400 µM) for 3 min. A selective decrease in the activity of succinate cytochrome c (complex II+III) could not be detected at a concentration of 100 µM of propofol. Only after increasing the concentration of propofol to 200 µM, an inhibition of the respiratory chain was seen. Instead of a selective decrease of complex II+III, a more generalized decrease was seen affecting the activities of complex I, complex II+III, complex III, and complex IV. Preincubation at a concentration of 400 µM of propofol also inhibited the

activity of complex II. The discrepancy between the results of the *in vivo* and *in vitro* experiments can be explained by a different incubation time of propofol and its different distribution between aqueous and membrane phase. In the *in vivo* tests, propofol was administered intravenously for a longer period (6 to 18 h), while in the *in vitro* tests, propofol was added to the reaction mixture only for 3 min during the preincubation period. In the *in vivo* experiments, it is likely that propofol, a compound with similar structure and characteristics to coenzyme Q, has been incorporated into the inner mitochondrial membrane where it was ready to accept the electrons generated by the complexes in the respiratory chain without transferring these to complex III. In the *in vitro* test, on the other hand, the contact of propofol with the sample was shorter, so one could assume that it was not fully incorporated into the inner mitochondrial membrane or its availability in the membrane phase was lower because it is more hydrophilic than coenzyme Q. Only when the concentration of propofol was doubled, an effect was seen on the respiratory chain but the inhibition was now not selective but generalized. Our results are in accordance with *in vitro* studies reported earlier in the literature.

To strengthen our *in vivo* findings, we have set up an *in vitro* experiment to test whether the addition of extra coenzyme Q to the homogenate was able to reduce the decline of the complex II+III activity after propofol incubation. As shown in figure 5, we found a significantly blunted decline in enzyme activity, reinforcing the hypothesis of a disturbing effect of propofol on the biologic function of coenzyme Q.

In an earlier study, we reported on a patient with Leber hereditary optic neuropathy who developed PRIS after administration of propofol for a period of 88 h by continuous infusion (total dose, 424 mg/kg; mean infusion dose, 4.8 mg kg^{-1} h^{-1}). This patient carried a pathogenic mutation in *MT-ND1* (m.3460G>A), a gene that codes for a subunit of complex I, which was also the cause of his blindness.[42] Although clinically he had no signs of myopathy or hepatopathy, the existence of a previous defect in complex I can explain why he was more vulnerable to the administration of propofol. In the literature, additional patients have been reported who developed PRIS after relatively lower doses of propofol and who were ultimately found to have a preexisting defect in the respiratory chain.[43,44] This explains why in some patients who survived PRIS severe catalytic defects have been detected in complex I or in complex IV, and not in
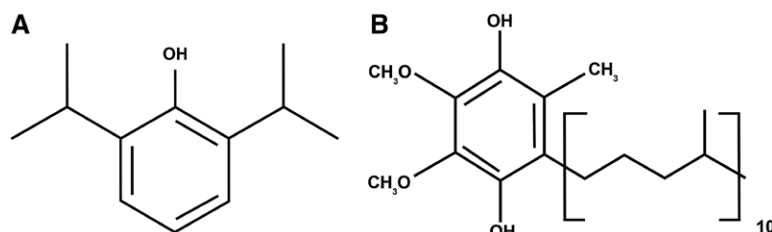

Fig. 7. Comparison between the molecular structure of propofol (*A*) and ubiquinol (reduced form of coenzyme Q) (*B*).

complex II+III. Not all patients with preexisting respiratory chain defect developed PRIS as uneventful sedation with propofol has been reported in such patients.[45] In the patients who are not carriers of a respiratory chain defect, larger doses of propofol are needed to interrupt the respiratory chain. We had the opportunity to examine postmortem tissues from a PRIS patient in the acute phase. Propofol had been administered to this patient for a prolonged period (>48 h). In skeletal muscle, the concentration of propofol was 10 µM. In this patient, indeed a decrease in the activity of complex II+III and of complex IV was detected, whereas the activities of the complexes II and III were normal when assayed individually. Also, the activity of complex I was normal.

Based on the results of *in vivo* and *in vitro* experiments in animals and on biochemical observations in a patient who died in the acute phase of PRIS, we can conclude that the main site of interaction of propofol with the respiratory chain is at the level of coenzyme Q. We hypothesize that after prolonged administration of high doses, propofol becomes incorporated into the inner mitochondrial membrane where it interrupts the electron flow in the respiratory chain mainly at the site of coenzyme Q. Interestingly, this effect could mostly be alleviated by supplementation with coenzyme Q, as shown in the *in vitro* experiment we have performed (fig. 5). An additional effect can be due to the interference of propofol with the cytochrome in complex IV (aa_3) and with cytochrome c. As it is possible to administer large doses of coenzyme Q intravenously to the patient, the administration of coenzyme Q could represent a potential preventive or therapeutic intervention, to be further investigated in the future. Future studies will be undertaken to test the protective effect of coenzyme Q.

Acknowledgments

Competing Interests

The authors declare no competing interests.

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