Possible Pathogenic Mechanism of Propofol Infusion Syndrome Involves Coenzyme Q

Arnaud Vincent Vanlander, M.D., Juergen Guenther Okun, Ph.D., Annick de Jaeger, M.D., Joël Smet, B.A.Sc., Elien De Latter, B.A.Sc., Boel De Paepe, Ph.D., Georges Dacremont, Ph.D., Birgitte Wuyts, M.D., Ph.D., Bert Vanheel, Ph.D., Peter De Paepe, M.D., Ph.D., Philippe Germaine Jorens, M.D., Ph.D., Niels Van Regenmortel, M.D., Rudy Van Coster, M.D., Ph.D.

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 aa3, 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)

PROPOFOL 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

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal’s Web site (www.anesthesiology.org).

Submitted for publication January 27, 2014. Accepted for publication September 4, 2014. From the Department of Pediatrics, Division of Pediatric Neurology and Metabolism (A.V.V., J.S., E.D.L., B.D.P., R.V.C.), Department of Critical Care Medicine, Division of Pediatric Intensive Care Medicine (A.d.J.), Department of Clinical Chemistry (B.W.), Department of Emergency Medicine (P.D.P.), Ghent University Hospital, Ghent, Belgium; Department of General Pediatrics, Division of Inherited Metabolic Diseases, University Children’s Hospital, Heidelberg, Germany (J.G.O.); Department of Pediatrics, University of Ghent, Ghent, Belgium (G.D.); Physiology Group, Department of Basic Medical Sciences, Ghent University, Ghent, Belgium (B.V.); Department of Critical Care Medicine, Antwerp University Hospital, Antwerp University, Edegem, Belgium (P.G.J., N.V.R.); and Department of Critical Care Medicine, ZNA Antwerp, Belgium (N.V.R.).

Copyright © 2014, the American Society of Anesthesiologists, Inc. Wolters Kluwer Health, Inc. All Rights Reserved. Anesthesiology 2015; 122:343-52

Anesthesiology, V 122 • No 2 343 February 2015
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.7 Also, in rat synaptosomes, propofol was shown to decrease $\Delta \Psi$ at concentrations between 30 and 100 µM.7 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 aa3.8 These findings argue against the hypothesis that propofol acts as uncoupler. Kam et al.5 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 adenine 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 Gezondheidszak, 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 Sirec 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 H2O; positive end-expiratory pressure, 3 cm H2O; 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, Cheshire, 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. 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 (5,600×g) 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., succinate reductase according to Rustin et al., succinate coenzyme Q reductase (complex II) according to Hatefi and Stiggall, succinate cytochrome c reductase (complex II+III activity) according to Sottocasa et al., cytochrome c ubiquinol reductase (complex III) according to Birch-Machin et al., cytochrome c oxidase (complex IV) according to Dimauro et al., and citrate synthase (CS) according to the protocol of Srere. 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.

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. 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.

**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. 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.

**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 and Smet et al. High-resolution clear native-PAGE was performed according to the methodology described by Wittig et al.

**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. 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 1,500g 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-Umstadt, 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% H2O, 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 (λex = 276 nm, λem = 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.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931937/ on 04/10/2017)
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 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, 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.
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 not added to the reaction mixture.

**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.
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 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. 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 aa3 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. Propofol is an even smaller molecule with high lipophilic character resulting in higher membrane solubility. Propofol is also known to be a potent antioxidant. 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. 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 cytochrome aa3. As shown earlier by Schenkman and Yan, perfusion of heart muscle with propofol resulted in 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. 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. 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. 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 adminis-
tered 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 pro-
longed administration of high doses, propofol becomes incor-
porated 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 addi-
tional effect can be due to the interference of propofol with
the cytochrome in complex IV (aa₃) and with cytochrome c.
As it is possible to administer large doses of coenzyme Q in-
travenously to the patient, the administration of coenzyme Q
could represent a potential preventive or therapeutic interven-
tion, to be further investigated in the future. Future studies
will be undertaken to test the protective effect of coenzyme Q.

Acknowledgments
The authors thank Vera Schulmann, B.A.Sc. (Department of
General Pediatrics, Division of Inherited Metabolic Diseases,
University Children’s Hospital, Heidelberg, Germany), for
her excellent technical assistance. The authors also thank Roos Colman, M.D. (Biostatistics Unit, Ghent University,
Ghent, Belgium), for her assistance in statistical analyses.

Supported by funding from the Fund for Scientific
Research (FWO, Fonds voor Wetenschappelijk Onderzoek,
Brussels, Belgium; grant nos. G.0066.06 and 3G020010).

Competing Interests
The authors declare no competing interests.

Correspondence
Address correspondence to Dr. Van Coster: Department of
Pediatrics, Division of Pediatric Neurology and Metabolism,
Ghent University Hospital, De Pintelaan 185, 9000 Ghent,
Belgium. rudy.vancoster@ugent.be. Information on purchas-
ing reprints may be found at www.anesthesiology.org or on
the masthead page at the beginning of this issue. ANESTHESIO-
LOGY’s articles are made freely accessible to all readers, for
personal use only, 6 months from the cover date of the issue.

References
1. Thompson KA, Goodale DB: The recent development of pro-
S400–4
2. Adverse effects of propofol (Diprivan). Propofol (Diprivan)
bivirkninger. Ugeskr Laeger 1990; 152:1176
Anaesth 1998; 8:491–9
4. Branca D, Roberti MS, Vincenti E, Scutari G: Uncoupling
effect of the general anesthetic 2,6-diisopropylphenol in iso-
lated rat liver mitochondria. Arch Biochem Biophys 1991;
290:517–21
5. Bains R, Moe MC, Vinje ML, Berg-Johnsen J: Sevoflurane and
propofol depolarize mitochondria in rat and human cere-
brocortical synaptosomes by different mechanisms. Acta
Anaesthesiol Scand 2009; 53:1354–60
6. Rigolet M, Devin A, Ayéret N, VANDAIS B, Guérin B:
Mechanisms of inhibition and uncoupling of respiration in iso-
lated rat liver mitochondria by the general anesthetic
7. Cray SH, Robinson BH, Cox PN: Lactic acidemia and brady-
arrhythmia in a child sedated with propofol. Crit Care Med
1998; 26:2087–92
8. Schenkman KA, Yan S: Propofol impairment of mitochon-
drial respiration in isolated perfused guinea pig hearts
determined by reflectance spectroscopy. Crit Care Med 2000;
28:172–7
2007; 62:690–701
10. Ypsilantis P, Politou M, Mikroulis D, Pitiakoudis M, Lam-
bropoulou M, Tsigalou C, Didiliς V, Bougioukas G,
Papadopoulos N, Manolas C, Simopoulos C: Organtoxicity
and mortality in propofol-sedated rabbits under prolonged
olism disturbances and AMPK activation in prolonged pro-
pofof-sedated rabbits under mechanical ventilation. Acta
Pharmacol Sin 2012; 33:27–33
acid oxidation in propofol infusion syndrome. Lancet 2001;
357:606–7
toxicity: Further evidence for a causal mechanism. Paediatr
Anaesth 2004; 14:505–8
15. Baumeister FA, Oberhoffer R, Liebhaber GM, Kunkel J,
Eberhardt J, Holthausen H, Peters J: Fatal propofol infusion
syndrome in association with ketogenic diet. Neuropediatrics
2007; 38:250–2
16. Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE,
Johnson DM, Strauss AW, Comeau AM, Eaton RB, Grady GF:
Tandem mass spectrometric analysis for amino, organic, and
fatty acid disorders in newborn dried blood spots: A two-
year summary from the New England Newborn Screening
17. Scholte HR, Ross JD, Blom W, Boonman AM, van Diggelen OP,
HALL CL, Huijmans JG, Luyt-Houwen IE, Kleijer WJ, de Klerk
JB, Przyrembel H, Verduin MH, Verstegen JC: Assessment of
deficiencies of fatty acyl-CoA dehydrogenases in fibroblasts,
WF, Sengers RC, Stadhouders AM, ter Laak HJ, Trijbels JM,
Veerkamp JH: A mitochondrial encephalomyopathy: The first
case with an established defect at the level of coenzyme Q.