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ORIGINAL ARTICLE

Isoflurane anesthetic hypersensitivity and progressive respiratory depression in a mouse model with isolated mitochondrial complex I deficiency

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Abstract

Background Children with mitochondrial disorders are frequently anesthetized for a wide range of operations. These disorders may interfere with the response to surgery and anesthesia. We examined anesthetic sensitivity to and respiratory effects of isoflurane in the Ndufs4 knockout (KO) mouse model. These mice exhibit an isolated mitochondrial complex I (CI) deficiency of the respiratory chain, and they also display clinical signs and symptoms resembling those of patients with mitochondrial CI disease.

Methods We investigated seven Ndufs4$^{-/-}$ knockout (KO), five Ndufs4$^{+/-}$ heterozygous (HZ) and five Ndufs4$^{+/+}$ wild type (WT) mice between 22 and 25 days and again between 31 and 34 days post-natal. Animals were placed inside an airtight box, breathing spontaneously while isoflurane was administered in increasing concentrations. Minimum alveolar concentration (MAC) was determined with the bracketing study design, using the response to electrical stimulation to the hind paw.

Results MAC for isoflurane was significantly lower in KO mice than in HZ and WT mice: 0.81 % $\pm$ 0.01 vs 1.55 $\pm$ 0.05 % and 1.55 $\pm$ 0.13 %, respectively, at 22–25 days, and 0.65 $\pm$ 0.05 %, 1.65 $\pm$ 0.08 % and 1.68 $\pm$ 0.08 % at 31–34 days. The KO mice showed severe respiratory depression at lower isoflurane concentrations than the WT and HZ mice.

Conclusion We observed an increased isoflurane anesthetic sensitivity and severe respiratory depression in the KO mice. The respiratory depression during anesthesia was strongly progressive with age. Since the pathophysiological consequences from complex I deficiency are mainly reflected in the central nervous system and our mouse model involves progressive encephalopathy, further investigation of isoflurane effects on brain mitochondrial function is warranted.

Keywords Anesthetics volatile · Isoflurane · Anesthesia depth · Mitochondrial disease · Complex I deficiency · Ndufs4 knockout mice

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Introduction

Mitochondria produce the energy requirements of cells, adenosine triphosphate (ATP), mainly through the reduction and oxidation reactions of the electron transfer chain (ETC) and oxidative phosphorylation (OXPHOS). The OXPHOS pathway consists of five multi-subunit enzyme complexes (complexes I–V) and two electron carriers (cytochrome C and coenzyme Q).

Mitochondrial disorders are most frequently due to defects in OXPHOS, and are genetically and phenotypically a heterogeneous group of disorders having an incidence of 1 in 10,000 live births [1].

The onset of symptoms may vary from birth to late adulthood. Organs that are highly energy-dependent such as the brain, heart and skeletal muscle are most vulnerable to mitochondrial defects. Since a treatment to correct this is not yet available, therapy is purely empirical and is still not very successful [2].

Children with mitochondrial disease are frequently anesthetized for diagnostic muscle biopsy, but also for a wide range of other procedures. These children often have several signs and symptoms (encephalomyopathy, respiratory and cardiac compromise, difficulty in swallowing and regurgitation), which may increase the risk associated with anesthesia and surgery [3]. Patients with mitochondrial disease resulting from a variety of defects in the respiratory chain and oxidative phosphorylation vary in their response to anesthetics and possibly in their peri-operative risk [3, 4].

The most frequently occurring defect in the OXPHOS system in humans is an isolated complex I (NADH:ubiquinone oxidoreductase) deficiency [5]. Most children suffering from complex I deficiency develop symptoms during their first year of life, and show rapid deterioration [2]. The majority of children with complex I deficiency present with Leigh or Leigh-like syndrome, which involves psychomotor retardation, brainstem dysfunction, seizures, failure to thrive, muscular hypotonia, abnormal eye movements and lactic acidosis [6].

The volatile anesthetic isoflurane inhibits complex I enzymatic rates [7]. In the nematode *Caenorhabditis elegans*, animals with a complex I deficiency are hypersensitive to volatile anesthetics [7]. In a clinical study, Morgan et al. [4] observed profound hypersensitivity to volatile anesthetics in a subset of children with defects in complex I function, requiring very low doses of sevoflurane to reach a bispectral index (BIS) value of 60. However, BIS monitoring, used in that study, is not a reliable index of anesthetic sensitivity in children who have central nervous system dysfunction [8].

The Ndufs4 subunit plays an important role in the assembly or stability of complex I and mutations in the *Ndufs4* gene, located on chromosome 5, cause a reduced complex I content as detected by blue native gel electrophoresis (BN-PAGE) and enzymatic activity measurements [9]. Patients harboring mutations in this gene show a Leigh or Leigh-like syndrome with death occurring at a very young age [6, 10]. The clinical signs and symptoms of Ndufs4 KO mice show extensive similarities to those of patients with mitochondrial complex I disease [9, 11]. The aim of this study was to investigate the anesthetic sensitivity and respiratory response to exposure of a volatile anesthetic agent, isoflurane, in the Ndufs4 KO mouse model at two stages, before and after the onset of severe symptoms.

Methods

All experiments were approved by the Regional Animal Ethics Committee (Nijmegen, the Netherlands) and performed under the guidelines of the Dutch Council for Animal Care. All efforts were made to reduce animal suffering and number of animals used in this study.

Animals

To determine isoflurane sensitivity in our experimental model, studies were conducted using Ndufs4$^{-/-}$ knockout (KO) and Ndufs4$^{+/-}$ heterozygous (HZ) mice, and their wild type (WT) Ndufs4$^{+/+}$ littermates (mixed 129/Sv: C57BL6 background), $n = 7$, $n = 5$, and $n = 5$, respectively. Mice were bred locally from heterozygous parent mice. The genotype was confirmed by polymerase chain reaction testing. Both male and female mice were included. Mice had ad libitum access to food and water and were fed on a standard animal diet (Ssniff GmbH, Soest, 76. Germany, V1534-300 R/M-H). Animals were group housed at 22 °C and were maintained on a day and night rhythm of 12 h.

Experimental design

Measurements took place when the mice were between postnatal (PN) 22–25 days (experiment I) before the pathophysiological symptoms appeared in the KO mice, and again between PN days 31–34 (experiment II) when Ndufs4 KO mice started to develop pathophysiological symptoms but before major inconvenience, as described by Kruse et al. [9], occurred. Weights and symptoms were recorded daily.

Each mouse was individually placed inside an airtight Plexiglas chamber (0.20 l), breathing spontaneously. Isoflurane (TEVA Pharmachemie, Haarlem, the Netherlands) was administered using an IsoTec-5 Isoflurane Anesthesia Vaporizer (Datex-Ohmeda, GE healthcare) with 33 % oxygen in air at a total flow rate of 0.5 l/min. The concentrations of isoflurane and CO_2 were measured continuously at the exhaust port of the chamber using a Capnomac-Ultima monitor (Datex, Helsinki). CO_2 levels in the chamber remained unmeasurably low throughout all experiments, proving lack of rebreathing.

Anesthetic endpoints mostly used in mice are the loss of righting reflex (LORR), which is used as a marker of unconsciousness/hypnosis and the withdrawal reflex to tail clamping or electrical pedal stimulation, as a measure of immobilization [12].

We used the withdrawal reflex to determine the MAC [13]. We used the withdrawal reflex to hind paw electrical

stimulation, not tail clamping, to determine immobilization, because, in pilot experiments, it gave more reliable and reproducible responses without any local noxious effects. In rats it gave comparable MAC values when compared to tail clamping [14]. Concentrations of isoflurane measured in the chamber, after equilibration of 6 min, were used as a substitute for alveolar concentrations in determining MAC.

The first experiment was performed when the mice were between PN 22–25 days. Based on pilot experiments, isoflurane was administered at 1.5 % in WT and HZ mice and 1.0 % in KO mice. The time to loss of mobility (LOM) and time to loss of righting reflex (LORR) were measured. LOM was reached when a mouse remained immobile for 60 s. LORR was defined as the lack of the ability to return to recumbency within 60 s after tilting the box 90°. Concentrations of isoflurane in the box at these time points were recorded. When anesthetized, a rectal temperature probe, ECG electrodes, and stimulation electrodes to the hind paw were applied. The eyes were lubricated. Animal breathing was clinically assessed by monitoring thoracic movement and by recording respiratory rate. Two experienced anesthesiologists counted the respirations during 15-s epochs. Temperature was maintained at 36–38 °C, using a heating lamp and warming pad.

Isoflurane was increased stepwise at regular intervals with an equilibration time of 6 min. After equilibration, the response to electrical stimulation (pulse intensity 4 mA, pulse duration 4 ms, train duration 500 ms, frequency 100 Hz (interval 10 ms) of the hind paw was recorded. Purposeful movement of the head and/or legs was considered a response [15]. When a response was noticed, the anesthetic concentration was increased stepwise with 0.2 % increments until the response was lost. When this point was reached the anesthetic concentration was decreased until there was a return of response [16, 17]. The MAC was determined as the average concentration of isoflurane at loss and return of response to pedal electrical stimulation.

After the MAC was determined, and after removal of electrodes, isoflurane was reset to 1 % in KO, and 1.5 % in WT and HZ mice. After equilibration, isoflurane was discontinued and the time till the return of righting reflex (RORR) and return of ambulation (ROA) were recorded. Total anesthesia duration (TAD) was also recorded.

After the first anesthetic depth determinations were performed between PN 22–25 days, the animals were allowed to fully awaken, and were returned to their housing. A similar experiment was performed when the mice were between PN 31–34 days.

After the end of the second experiment the animals were anesthetized again with a lower isoflurane concentration than the MAC to obtain an arterial blood gas sample, taken directly from the abdominal aorta. In some cases puncture of the abdominal aorta caused a hemorrhage that was aspirated for lactate determination. Arterial blood gas analysis was performed using the i-STAT® System (Abbott Point of Care Inc, Princeton, USA). Subsequently the animals were sacrificed by cervical dislocation.

Statistical analysis

Statistical analysis was performed using Prism 5 (GraphPad Software Inc., La Jolla, Ca, USA). Comparisons between the three groups were performed using the non-parametric Kruskal–Wallis test as a Gaussian distribution was not observed. Comparisons between groups were performed using the non-parametric Mann–Whitney test. The Pearson test was used to assess the correlation between the MAC values and the decrease in respiratory rate at the MAC in KO vs WT mice. All data are presented as mean ± standard error of the mean (SEM). Significance was defined as $P < 0.05$.

Results

There were no statistically significant differences in age in both the first and second experiment between all groups. There were no differences in weight between HZ and WT mice. KO mice weighed significantly less (12.3 ± 0.71 g), in the second experiment, than WT (15.3 ± 0.60 g) and HZ mice (17.1 ± 1.30 g), $P < 0.02$ (Table 1). Despite being of the same age, the KO mice were clinically clearly distinguishable by their smaller size and hair loss.

During the first experiment the maximum (baseline) respiratory rate of KO mice, 130 ± 7.5 breaths/min, did not differ from WT (130 ± 6.3 breaths/min) and HZ (135 ± 10.7 breaths/min) mice. At the more advanced age, the KO mice showed a lower maximum (baseline) respiratory rate (83 ± 10.5 breaths/min) ($P < 0.001$), than WT and HZ mice (139 ± 2.3 breaths/min, and 157 ± 3.4 breaths/min respectively (Table 2). During increasing isoflurane concentration there was progressive slowing of the respiratory rate. The maximal decrease in respiratory rate at MAC concentrations was greater in KO mice. Also, signs of airway obstruction were more frequent at higher isoflurane concentrations. Both the baseline and the minimal respiratory rates at reaching the MAC were significantly lower in KO mice in experiment II than I. During the second experiment, one KO mouse died of apnea. Respiratory depression caused by isoflurane exposure was quickly reversible by lowering the isoflurane concentration. Although we started at lower isoflurane concentrations in KO mice, they had a significantly shorter onset time of

Table 1 Age and weight

		WT	HZ	KO
Age	I	23.6 ± 0.51	23.2 ± 0.58	23.3 ± 0.18
	II	32.8 ± 0.58	32.6 ± 0.51	32.7 ± 0.52
Weight	I	9.6 ± 0.77	9.5 ± 0.41	7.8 ± 0.48
	II	15.3 ± 0.60	17.1 ± 1.30	12.3 ± 0.71*

WT = *Ndufs4*$^{+/+}$ mice ($n = 5$), HZ = *Ndufs4*$^{+/-}$ mice ($n = 5$), KO = *Ndufs4*$^{-/-}$ mice ($n = 7$). Age in days, weight in grams

All data are mean ± SEM

I experiment I, *II* experiment II

* Statistical significance

Table 2 Clinical features during isoflurane anesthesia

		WT	HZ	KO
Temp	I	37.2 ± 0.21	36.6 ± 0.12	36.9 ± 0.10
	II	37.3 ± 0.17	36.5 ± 0.24	36.5 ± 0.31
HR	I	273 ± 12.4	268 ± 10.9	294 ± 5.7
	II	286 ± 11.3	297 ± 8.9	306 ± 5.3
Baseline RR	I	130 ± 6.3	135 ± 10.7	130 ± 7.5
	II	139 ± 2.3	157 ± 3.4	83 ± 10.5*
Δ RR	I	44 ± 5.5	52 ± 13.4	85 ± 8.4*
	II	70 ± 5.6	83 ± 8.9	54 ± 12.2

WT = *Ndufs4*$^{+/+}$ mice ($n = 5$), HZ = *Ndufs4*$^{+/-}$mice ($n = 5$), KO = *Ndufs4*$^{-/-}$ mice ($n = 7$)

All data represent mean ± SEM

Temp mean temperature in °C, *HR* mean heart rate in beats/min, *Max RR* maximum or baseline respiratory rate in breaths/min, *Δ RR* maximal decrease in respiratory rate in breaths/min, *I* experiment I, *II* experiment II

* Statistical significance

anesthesia, measured by time until LOM ($P < 0.02$) and LORR ($P < 0.02$), and at lower isoflurane concentrations ($P < 0.01$) when compared to WT and HZ mice in both the first and second experiment (Table 3). There were no significant differences between WT and HZ mice. Mean heart rate while being anesthetized with isoflurane was similar in all groups, in both experiment I and II (Table 2). The same was true for mean temperature during the experiment (Table 2).

Table 4 Minimum alveolar concentration of isoflurane

		WT	HZ	KO
MAC (%)	I	1.55 ± 0.13	1.55 ± 0.05	0.81 ± 0.01*
	II	1.68 ± 0.08	1.65 ± 0.08	0.68 ± 0.05*

WT = *Ndufs4*$^{+/+}$ mice ($n = 5$), HZ = *Ndufs4*$^{+/-}$ mice ($n = 5$), KO = *Ndufs4*$^{-/-}$ mice ($n = 7$). All data are mean (SEM)

MAC minimum alveolar concentration of isoflurane, *I* experiment I, *II* experiment II

* Statistical significance

The MAC in KO mice (0.81 ± 0.01 %) was significantly lower than in both HZ (1.55 ± 0.05 %) and WT (1.55 ± 0.13 %) mice ($P < 0.003$), in the first experiment (Table 4). In the second experiment, MAC could not be determined in three mice because of severe respiratory depression, before a loss of response to the stimulus was reached at isoflurane concentrations of 0.70 %. One of these mice died as a consequence of respiratory depression. The mean MAC in experiment II for KO mice was 0.65 ± 0.05 %, HZ 1.65 ± 0.08 % and WT 1.68 ± 0.08 %, ($P < 0.02$) (Table 4). There was no difference in MAC between HZ and WT mice in both experiments (Fig. 1). The correlation between the MAC and decrease of respiratory rate from baseline is shown in Fig. 2. There was a significant correlation between MAC of isoflurane and the decrease in respiratory rate in the KO mice, but not in the WT mice.

Only in the first round of experiments did KO mice demonstrate a faster return of righting reflex (RORR) than

Table 3 Loss of mobility and righting reflex

		LOM		LORR	
		Time (s)	Isoflurane (%)	Time (s)	Isoflurane (%)
Experiment I	WT	85.2 ± 11.5	1.18 ± 0.05	160.2 ± 15.1	1.34 ± 0.03
	HZ	95.8 ± 15.6	1.27 ± 0.07	146.8 ± 17.7	1.38 ± 0.03
	KO	57.3 ± 3.0*	0.74 ± 0.06*	99.0 ± 7.8*	0.84 ± 0.05*
Experiment II	WT	121.6 ± 18.9	1.32 ± 0.003	179.8 ± 23.7	1.37 ± 0.01
	HZ	106.0 ± 14.3	1.22 ± 0.007	165.2 ± 14.4	1.34 ± 0.03
	KO	51.6 ± 5.6*	0.76 ± 0.03*	86.1 ± 8.0*	0.87 ± 0.02*

WT = *Ndufs4*$^{+/+}$ mice ($n = 5$), HZ = *Ndufs4*$^{+/-}$ mice ($n = 5$), KO = *Ndufs4*$^{-/-}$ mice ($n = 5$). All data are mean ± SEM

LOM loss of mobility, *LORR* loss of righting reflex

* Statistical significance

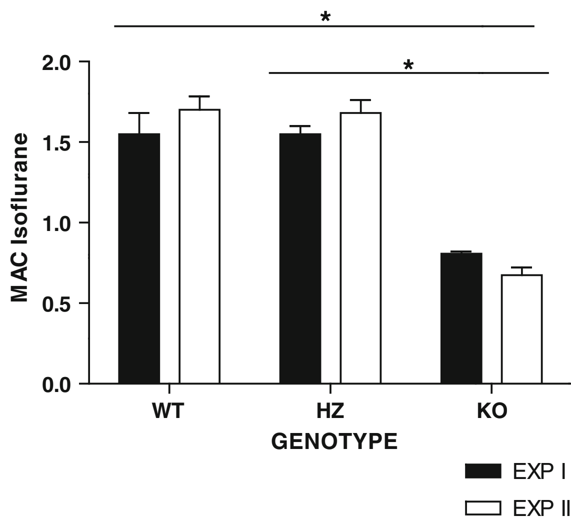

Fig. 1 Minimum alveolar concentrations (MAC) of isoflurane are reported for WT = *Ndufs4*$^{+/+}$ ($n = 5$), HZ = *Ndufs4*$^{+/-}$ ($n = 5$) and KO = *Ndufs4*$^{-/-}$ ($n = 7$) in EXP I = experiment I and EXP II = experiment II. *Bars* represent mean MAC for WT, HZ, and KO mice. *Error bars* show the standard error of the mean. The values for the KO mice were significantly different from those for WT and HZ mice, in both experiment I and II. *$P < 0.005$

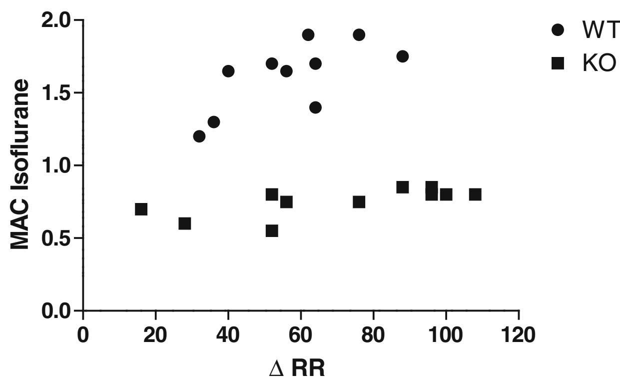

Fig. 2 Cumulative correlation plot between decrease in respiratory rate from baseline (ΔRR) vs minimum alveolar concentration (MAC) from experiment I and experiment II. Correlation was determined for WT = *Ndufs4*$^{+/+}$ ($n = 5$) and KO = *Ndufs4*$^{-/-}$ ($n = 7$). *Solid black circles* indicate the WT mice and *solid black squares* indicate the KO mice. There was no significant correlation between (ΔRR) vs MAC in the WT mice. However, a significant correlation was observed for the KO mice ($P = 0.019$)

WT and HZ mice, $P < 0.003$ (Table 5). In the second experiment we had to lower isoflurane concentrations rapidly in 6 out of 7 KO mice, because of severe respiratory depression. This could be a factor in the lack of difference in RORR in this experiment. There was no difference in time till return of ambulation (ROA) between groups (Table 5). In KO mice total anesthesia duration (TAD) was significantly shorter than in WT and HZ mice in both experiments (Table 5) due to the fact that in KO mice the MAC was achieved at lower concentrations, achieved in a shorter period of time.

Table 5 Recovery from anesthesia

		WT	HZ	KO
RORR (s)	I	567 ± 130.2	463 ± 52.5	105 ± 6.8*
	II	238 ± 50.8	239 ± 46.9	202 ± 42.3
ROA (s)	I	1,092 ± 95.2	1,233 ± 64.4	681 ± 225.0
	II	712 ± 159.7	702 ± 85.0	420 ± 30.4
TAD (min)	I	120 ± 9.6	110 ± 8.5	69 ± 4.1*
	II	118 ± 10.7	90 ± 5.8	42 ± 3.3*

WT = *Ndufs4*$^{+/+}$ mice ($n = 5$), HZ = *Ndufs4*$^{+/-}$ mice ($n = 5$), KO = *Ndufs4*$^{-/-}$ mice ($n = 5$). All data are mean ± SEM

RORR return of righting reflex, *ROA* return of ambulation, *TAD* total anesthesia duration

* Statistical significance

There were no statistically significant differences in KO mice between experiment I and II in onset and recovery times.

The results of blood gas analysis are presented in Table 6. There were no signs of hypoxemia or hypercarbia observed in either group. In two KO mice, lactate, determined in the blood aspirated from the hemorrhage that occurred with puncture of the abdominal aorta, was 15.6 and 15.6 mmol/l. These results clearly point to a metabolic acidosis due to lactic acidemia.

Discussion

The main result of our study was an increased isoflurane sensitivity in Ndufs4 knockout mice, compared to their wild type and heterozygous littermates. This involved both the righting reflex, considered as a marker of unconsciousness, and the withdrawal reflex to electrical hind paw stimulation, as a marker of immobilization, used to determine MAC. The MAC was slightly lower at the more advanced age (31–34 days) compared to the younger age (22–25 days). All our KO mice, except one that died from respiratory depression during the experiment, showed a fast and complete recovery from anesthesia when isoflurane exposure was terminated. Quintana et al. [18] recently reported almost identical data, with a 2.5- to 3-fold higher sensitivity in a tail clamp response to isoflurane and halothane at 23–27 days in Ndufs4 KO mice compared to WT. The higher sensitivity in their study could be explained by a different stimulation method, but also by the shorter equilibration times used in our study. Furthermore, a limitation of our study was that it was impossible to administer equal isoflurane concentrations to KO mice, since they got severe respiratory depression much faster. Anyway, this

Table 6 Arterial blood gas analysis at the end of experiments

	pH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	BE	HCO_3^- (mmol/l)	SaO_2 (%)	Lactate (mmol/l)
KO	7.02	192	37.4	−21.0	9.6	99	13.30
KO	7.00	*x*	28.7	−24.0	7.1	*x*	*x*
KO	7.09	178	30.2	−21.0	9.2	99	11.29
KO*				−27.0	4.1	98	15.58
KO*				−26.0	2.6	100	15.57
HZ	7.34	206	36.8	−5.0	20.2	100	3.35
HZ	7.38	215	27.8	−9.0	16.5	100	6.01
HZ	7.35	206	34.8	−6.0	19.3	100	4.16
HZ	7.44	302	26.1	−6.0	17.8	100	5.95
WT	7.34	191	37.9	−5.0	20.6	100	3.99
WT	7.42	215	33.2	−2.0	22.0	100	4.49
WT	7.40	213	26.3	−8.0	16.4	100	4.60
WT*					16.4	100	4.19

WT = *Ndufs4*$^{+/+}$ mice, HZ = *Ndufs4*$^{+/-}$ mice, KO = *Ndufs4*$^{-/-}$ mice

x no result

* Analysis after direct aspiration of hemorrhage from abdominal aorta

effect in complex I deficient mice cannot yet be extrapolated to children with complex I deficiency since a human study did not directly point to a specific hypersensitivity to volatile anesthetics [19]. Furthermore, in our KO mice there is a complete inactivation of the *Ndufs4* gene while in children with Ndufs4 mutations some residual activity of complex I is still present.

No mention was made about respiratory effects during anesthesia in the report of Quintana et al. [18]. We observed a strong, progressive, near fatal, but completely reversible, respiratory depression during isoflurane exposure in Ndufs4 KO mice. This occurred at concentrations well below the MAC for immobilization. Ndufs4 KO mice are known to develop progressive respiratory dysfunction i.e. bradypnea and apnea, leading to premature death [20]. Central, rather than peripheral, mechanisms are involved. Older awake KO mice in that study had lower breathing rates than controls, which was also observed in our study. There were no signs of hypoxemia or hypercarbia in all groups in the arterial blood gas analysis at the end, although this can be partly explained by the fact that all mice were allowed to recover from anesthesia before (briefly) being anesthetized again to obtain the arterial blood sample. However, it is likely that KO mice did develop hypercarbia, and possibly hypoxemia, caused by the severe respiratory depression at reaching MAC concentrations. Future investigations of anesthesia-induced respiratory depression in complex I deficient mice is warranted. Awaiting such data, it may be presumed that children with complex I deficiency are also at an increased risk of respiratory depression during anesthesia, even before a deep anesthetic phase is reached, and one should monitor ventilation carefully during and after administration of (volatile) anesthesia in case of (suspected) mitochondrial disease.

We did not observe significant differences in heart rate, using electrocardiographic monitoring, during isoflurane anesthesia between all groups. A previous study reported a lower heart rate for KO mice than for control mice, especially in late stages of the disease [20]. The heart rates in unanesthetized mice in that study were much higher than heart rates of both KO and control mice in our study during anesthesia.

Despite being used in millions of patients, the mechanism by which volatile anesthetics produce their effects is still scarcely known. The many endpoints associated with the general anesthetic state are probably the result of effects at different sites of action [21]. It was long assumed that they interact non-specifically with neuronal membranes, based on their lipophilicity [13]. However, at present general anesthetics are believed to exert their effects by binding to specific protein targets, including ligand-gated ion channels, such as $GABA_A$ receptors, NMDA receptors and a range of two-pore domain potassium channels [13, 22]. Since different classes of general anesthetics act on different spectra of receptors and channels it is unlikely that there is one common mechanism for all general anesthetics.

It has long been known that volatile anesthetics cause depression of mitochondrial function [23]. Halothane, isoflurane and sevoflurane inhibit oxidation of substrate at complex I in a dose-dependent fashion [24]. Inhalational anesthetics are also known to uncouple mitochondrial respiration from ATP generation [25]. They induce mitochondrial flavoprotein oxidation through the opening of adenosine triphosphate-dependent mitochondrial potassium (mitoK-ATP) channels [26].

Complex I malfunction causes several mitochondrial effects including reduced ATP production, changes in membrane depolarization, increased NADH levels,

increased production of ROS and lipid peroxidation, alterations in redox state, cellular calcium homeostasis, and changes in mitochondrial morphology [6, 27]. In *C. elegans* with defective function of complex I, there is hypersensitivity to volatile anesthetics causing reversible immobility [7]. Our study confirms these results are also true in a mammalian animal model. Although there is a clear correlation between complex I-dependent oxidative phosphorylation capacity and anesthetic sensitivity, it is not the absolute rate of complex I enzymatic activity which predicts the threshold for immobilization to volatile anesthetics [7]. At present it remains difficult to explain the exact link between complex I dysfunction and volatile anesthetic hypersensitivity [18].

Children with complex I deficiency have early onset neurological deterioration. Optimal complex I function and mitochondrial ROS production are necessary to maintain neuronal activity and synaptic transmission. Cellular ROS production is inversely correlated with CI activity and altered reactive oxygen species levels, as seen in human fibroblasts, and might have a central regulatory role in the pathology of CI deficiency [2]. ROS are routinely generated as by-products of the interaction between free electrons and oxygen and are an unavoidable consequence of aerobic metabolism. Mitochondria are also necessary for the generation of substances that eliminate free radicals formed during aerobic metabolism. If the production of ROS becomes too great to be counterbalanced by its antioxidant system, damage to proteins, lipids and DNA occurs. Sensitivity to volatile anesthetics may be related to both complex I-mediated impairment of OXPHOS and to oxidative damage to specific molecules [28]. It may be that certain specific proteins are damaged due to complex I deficiency and that the accumulation of these damaged molecules is responsible for the increase in anesthetic sensitivity [7, 28].

Volatile anesthetics also have a well-known protective effect on mitochondria, which is responsible for cardioprotection against ischemia and reperfusion via anesthetic preconditioning [29]. Volatile anesthetics have also been shown to have both neuroprotective and neurotoxic effects in animal models [30]. Neurotoxic effects of volatile anesthetics occur especially in the developing brain, and mitochondrial damage seems to be an important target of anesthesia-induced developmental neurodegeneration [31]. Volatile anesthetics may also cause neuroprotection by mild inhibition of respiratory chain complexes I–V and subsequent mitochondrial membrane depolarization [32]. It has been known since 1992 that isoflurane reduces excitatory synaptic transmission in the rat hippocampus [33].

In conclusion, there is obviously a broad range of complex biochemical and physiological aspects of complex I dysfunction. Since the pathology from complex I deficiency occurs mainly in the central nervous system [2], we stress the necessity of specific investigation of the effects of general anesthetics on brain mitochondrial function.

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Conflict of interests J Smeitink is CEO of Khondrion, a spin-off company of the Radboud University Medical Centre, Nijmegen, The Netherlands.

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