Published in final edited form as:

Brain Res. 2011 June 17; 1396: 69–76. doi:10.1016/j.brainres.2011.04.002.

Optically measured NADH concentrations are unaffected by propofol induced EEG silence during transient cerebral hypoperfusion in anesthetized rabbits[☆]

Mei Wang^a, Sachin Agarwal^b, Avraham Mayevsky^c, and Shailendra Joshi^{a,*}

^aDepartment of Anesthesiology, Columbia University, USA

^bDivision of Neuro-Critical Care, Columbia University, USA

^cThe Mina and Everard Goodman Faculty of Life Sciences and The Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Israel

Abstract

The neuroprotective benefit of intra-operative anesthetics is widely described and routinely aimed to invoke electroencephalographic (EEG) silence in anticipation of transient cerebral ischemia. Previous rat survival studies have questioned an additional benefit from achieving EEG silence during transient global cerebral hypoperfusion. Surgical preparation on twelve New Zealand white rabbits under ketamine–propofol anesthesia, included placement of skull screws for bilateral EEG monitoring, skull shaving for laser Doppler probes, and a 5 mm diameter right temporal craniotomy for the NADH probe. Transient global cerebral hypoperfusion was achieved with bilateral internal carotid artery occlusion and pharmacologically induced systemic hypotension. All animals acted as controls, and had cerebral hypoperfusion under baseline propofol anesthesia with an active EEG. Thereafter, animals were randomized to receive bolus injection of intracarotid (3–5 mg) or intravenous (10–20 mg) 1% propofol to create EEG silence for 1–2 min. The data collected at baseline, peak hypoperfusion, and 5 and 10 min post hypoperfusion was analyzed by repeated measures ANOVA with post hoc Bonferroni–Dunn test. Eleven of the twelve rabbits completed the protocol. Hemodynamics and cerebral blood flow changes were comparable in all the animals. Compared to controls, the increase in NADH during ischemia was unaffected by EEG silence with either intravenous or intraarterial propofol. We failed to observe any significant additional attenuation of the elevation in NADH levels with propofol induced EEG silence during transient global cerebral hypoperfusion. This is consistent with previous rat survival studies showing that EEG silence was not required for full neuroprotective effects of pentothal anesthesia.

Keywords

NADH; EEG silence; Propofol; Cerebral hypoperfusion

[☆]Funding support: NCI R01 Ca 12500 Grant, from the National Institute of Health, Bethesda, Maryland.

© 2011 Elsevier B.V. All rights reserved.

^{*}Corresponding author at: Department of Anesthesiology, PH 505, College of Physicians and Surgeons of Columbia University, 630 West 168th Street, New York, NY 10032, USA. Fax: +1 212 305 8287., sj121@columbia.edu (S. Joshi).

1. Introduction

It's well known that under anaerobic conditions NADH oxidation ceases and tissue concentrations of NADH rapidly increase. Moreover, NADH (and not NAD⁺) molecule absorbs light at 320–380 nm and emits fluorescent light at 420–480 nm (Fig. 1). These optical characteristics permit real-time measurement of mitochondrial NADH levels by ultraviolet spectroscopy (Mayevsky and Rogatsky, 2007). Previous studies suggested that transient global cerebral hypoperfusion (CHP) results in anaerobic metabolism and an increase in optically measured brain tissue NADH levels (Tomlinson et al., 1993a, 1993b). Furthermore, in the past we have observed that intraarterial injection of anesthetic drugs can produce EEG silence at a fraction of total systemic doses with preserved hemodynamics i.e. cerebral blood flow and mean arterial pressures (Wang et al., 2003).

Warner et al. (1996) first demonstrated that the reduction in infarct volume in rats subjected to focal ischemia was similar whether pentobarbital was administered in EEG-burst suppression doses or in doses approximately one-third of that required to produce EEG burst suppression. These data were confirmed in a similar study conducted by Schmid-Elsaesser et al. (1999). Pittman et al. (1997) demonstrated that neurologic and histologic outcomes were similar in pentobarbital- and propofol-anesthetized rats subjected to focal ischemia. Given that pentobarbital is considered to have neuroprotective properties, these data indirectly indicate that propofol can also reduce ischemic injury.

We hypothesized that compared to controls with an active EEG, propofol in doses sufficient to produce EEG silence prior to transient global cerebral hypoperfusion, will not significantly decrease tissue metabolism so as to attenuate the rise in brain tissue NADH concentrations. Furthermore, intraarterial administration compared to intravenous mode will demonstrate a greater attenuation in the increase in NADH levels due to less adverse effects on systemic and cerebral hemodynamics.

2. Results

The experimental protocol was completed by all twelve rabbits except one due to persistent hypotension and no recovery of mean arterial pressure after the first CHP challenge. The animal was excluded from the study. In the remaining 11 animals the baseline parameters were comparable between the three randomized groups. Transient cerebral hypoperfusion resulted in a significant reduction of mean arterial pressure (MAP), heart rate (HR), end tidal carbon dioxide (ETCO₂) and cerebral blood flow that was comparable between all three challenges, Fig. 2. There was a significant hyperemic reperfusion response in all three challenges at 5 min after CHP, unaffected by propofol induced EEG silence. Recovery of mean arterial pressure was delayed after intravenous propofol pretreatment, Table 1.

In all three groups transient CHP, resulted in a significant increase in NADH concentrations that promptly returned to baseline value as blood flow recovered, Fig. 3. Pretreatment with propofol in doses sufficient to cause EEG silence did not attenuate the relative increase in NADH concentration or the return to baseline concentrations once the blood flow was restored. Transient CHP did not result in any sustained changes in EEG activity in any challenge.

Real-time measurement of NADH concentration can help us plot the relationship in changes in NADH concentrations as a function of MAP or cerebral blood flow. Figs. 4a and b are representative plots of such a relationship during the three treatments in one animal. Since NADH and mean arterial pressure recover to near base-line values, the plot between NADH concentrations and MAP looks like a hysteresis loop, Fig. 4a. A similar loop can be generated to explain the relationship between NADH and % cerebral blood flow, Fig. 4b. However in this case we observed an overshoot due to hyperemic increase in CBF. In some animals transient negative overshoot of NADH was seen during hyperemia.

3. Discussion

In the brain tissue, at normal body temperature, synaptic transmission accounts for 50% of the brain tissue metabolism. Neuroprotective properties of anesthetic drugs are often attributed to their ability to suppress synaptic transmission. So, while it makes intuitive sense that EEG silence by anesthetic drugs will significantly attenuate the increase in NADH concentrations during transient cerebral hypoperfusion, the current study was unable to confirm it. The control measurements with low dose anesthesia and an active EEG preceded the intravenous or intraarterial pharmacological challenges, and the baseline hemodynamics along with subsequent magnitude of hypotension and the reduction in CBF during transient global hypoperfusion were comparable. However we did not observe any significant difference in the attenuation of NADH rise among any of the groups. Furthermore, contrary to our hypothesis, the intraarterial approach was not significantly different from intravenous delivery in the net increase in NADH during transient cerebral hypoperfusion. Similar results have been documented by other rat survival studies (Warner et al., 1996) in a focal ischemia model using sodium pentobarbital. That study questioned the reduction of cerebral metabolic rate (CMR) being the principal mechanism of neuroprotection by barbiturates, as they were unable to find a dose–response relationship between benefit obtained and EEG quiescence. They concluded that smaller doses of drug may be as efficacious as those required to substantively suppress electrocortical activity. These findings were further investigated by Schmid-Elsaesser et al. (1999) and showed that compared to controls, low-dose thiopental significantly decreased infarct volume by 28% and high-dose thiopental by 29%. The results indicated that barbiturates provide cerebral protection but that the magnitude of this effect has been overestimated. Furthermore, it is conceivable that the peak effect of such protective mechanisms does not parallel the reduction in CMR and other mechanisms than CMR reduction seem to contribute to their beneficial effects. Our study results agreed with the studies described above using a novel way of monitoring cerebral metabolic rate in real time.

We demonstrated that it is feasible to monitor brain tissue NADH concentrations with ultraviolet spectroscopy through the dura mater. As mentioned before, energy generation by the mitochondria is a complex process closely linked to the metabolic needs of the cell (Kasischke et al., 2004). Oxidation of NADH generates vast amounts of energy that maintains the polarity across the inner mitochondrial membrane and the resulting influx of hydrogen ions leads to the generation of ATP. This ATP in the mitochondria is then transferred to the cytosol via ATP–ADP translocase reaction. The generation of ATP is tightly controlled in the tissue by the metabolic rate. The tissue ADP level plays a critical

role in the feedback control of glycolysis and citric acid cycle. Increasing ADP levels in the cell increase glycolysis, thereby the amount of NADH. In aerobic condition NADH is oxidized to generate ATP. If tissue metabolism is suppressed then the rate of glycolysis will decrease with a corresponding decrease in the rate of NADH synthesis (Stryer, 1988). As we mentioned before, the NADH (and not NAD^+) molecule absorbs light at 320–380 nm and emits fluorescent light at 420–480 nm. These optical characteristics permit real-time measurement of mitochondrial NADH levels by ultraviolet spectroscopy (Mayevsky and Rogatsky, 2007). Therefore, measuring NADH concentration provides a means to explore the relationship between cerebral metabolism and blood flow. Most of the fluorescence signal originates from the bound NADH in the respiratory chain. We (Mayevsky and Rogatsky, 2007) along with other investigators (Avi-Dor et al., 1962) came to the same conclusion that the average fluorescence yield of reduced pyridine nucleotide in mitochondria is 6 to 8 times the yield of NADH and NADPH in aqueous solution. Furthermore, the yield of mitochondrial NADH is substantially higher than the yield of mitochondrial NADPH. This ability to affect the redox state of brain tissue could provide insights into the neuroprotective potential of various drugs or interventions like hypothermia.

Our study also tried to evaluate the feasibility of real time monitoring of NADH concentrations and cerebral blood flow expressed as NADH–CBF hysteresis loops shown in Fig. 4b. We believe these loops represent the ability of the tissue to adapt to a given level of ischemia and thus could help understand the neuroprotective effects of the drugs. We found that for any given rate of cerebral blood flow the NADH concentration in the tissue was lower at the onset of ischemia compared to reperfusion phase. The inference is that at the onset of tissue ischemia, there is probably a greater adaptability to the reduction in blood flow compared to the reperfusion phase even at a constant level of blood flow. For future studies, we hypothesize that for a perfect neuroprotective drug or an intervention such as deep hypothermia, the rise in NADH will be significantly attenuated and would bear similar relationship with CBF during ischemia and reperfusion. In neuroprotective drugs that act only via diminution in synaptic transmission, the rise in NADH would only be partly attenuated (Fig. 5). Further projecting the concept of flow NADH, we anticipate that with sustained ischemia and neural injury, NADH level would not return to baseline and the NADH–CBF hysteresis loop will remain open. Further investigations are needed to determine the relationship between increase in brain tissue NADH concentrations and evolution of focal neurological injury (Joshi et al., 2008).

Our study faces several limitations, particularly the measurement of NADH in the brain tissue which can be affected by a number of factors. (i) Tissue movement relative to the probe, both mechanical and due to brain edema. (ii) Shift of fluid into-intracranial and extracranial compartments such as during cortical spreading depression. (iii) Changes in concentrations of tissue hemoglobin that absorbs the light signals. (iv) Substrate availability for glycolysis. We took precautions to prevent any movement, ensured adequate depth of anesthesia by carefully monitoring the EEG and measured cerebral blood flow with laser Doppler in real-time. Therefore, these potential sources of errors in NADH measurements, if any, were shared by all three challenges (Mayevsky and Rogatsky, 2007; Rampil et al., 1992).

Previously, we have attempted to differentiate the physiological rise in NADH with pathological situations. The responses of mitochondrial NADH to the entire range of experimental and clinical conditions have been described in a recently published review article (Mayevsky and Rogatsky, 2007) using the same technique as in this study. We have seen that the rise in NADH levels is tightly correlated with the reduction in MAP. Mild changes in MAP will be in the physiological range as compared to hemorrhagic shock where the reduction in MAP is severe and the NADH rise will be in the pathological range. Similarly, the cortical spreading depression event could either be a physiological or a pathological event. Our technology is limited to the tissue below the probe. Nevertheless, we believe that monitoring of NADH is the only way to monitor mitochondrial function in vivo. The aim of the present study was to monitor the cortex under the specific conditions defined in the protocol. The results may not reflect the events occurring in other structures of the brain.

We would also like to mention that NADH rise may not reach its peak with CBF being 10% of the baseline levels. The relationship between CBF and NADH is not linear as described by us (Mayevsky and Rogatsky, 2007) and others (Paschen et al., 1992).

Furthermore, since the experiments were conducted in anesthetized animals, it could be argued that the background anesthetic-propofol infusion rate was excessive and the additional doses of propofol were so small that they were unlikely to attenuate the increase in NADH. The background dose of propofol 200–400 $\mu\text{g/kg/min}$ was greater than the hypnotic dose range in human subjects. However, we carefully controlled the level of anesthesia by monitoring EEG activity. In rabbits, a decrease in dose below this level of propofol infusion produces a high frequency low amplitude arousal pattern on the EEG with clinical correlates of increased respiratory effort or small movements in the limbs or the tongue. In this study, we also found that the rise in NADH concentration due to bolus propofol was only minimal (7–10%) when mean arterial pressure was maintained with phenylephrine (Fig. 6). Therefore we conclude that background anesthetics in the absence of any hypotension did not affect the NADH concentrations. The baseline hemodynamic parameters i.e. HR and MAP were in the normal range and did not indicate excessive doses of background anesthesia.

In conclusion, our failure to observe any additional attenuation in the rise of NADH during transient global cerebral hypoperfusion at propofol doses causing EEG silence has very significant implications. We infer that propofol induced suppression of brain metabolism to the extent of EEG silence does not prevent the progression of ischemia in anesthetized rabbits during transient global cerebral hypoperfusion. The current study is the first attempt towards many to call into question the routine practice of deepening the level of anesthesia during surgery in anticipation of cerebral ischemia.

4. Experimental procedures

After IACUC approval and in accordance with the guidelines for the care of laboratory animals, experiments were conducted on New Zealand white rabbits (3–4 lb in weight). The animals were anesthetized with intramuscular ketamine until an intravenous line was

secured and an infusion of propofol was initiated. Surgical preparation included ear vein cannulation, placement of a femoral arterial line, tracheostomy, and isolation of the carotid arteries (Joshi et al., 2004). EEG leads were secured to the skull by stainless steel screws. The skull was carefully milled, until cerebral blood vessels could be seen through the inner table of the skull and was left intact. Laser Doppler probes were secured to the skull using small plastic retainers. Heart rate, EKG, oxygen saturation, respiratory rate and end-tidal CO₂, and the esophageal temperature were continuously monitored. A small thermistor probe was placed on the dura mater to record the brain surface temperature. The data were collected on a 16 channel Power Lab data collection system. The level of propofol anesthesia was carefully titrated to achieve EEG activity in the 100–200 μ V range with both high frequency–low amplitude and low frequency–high amplitude activities.

4.1. Transient global cerebral hypoperfusion

Transient cerebral ischemia was induced by bilateral carotid occlusion and intravenous injection of 20–30 mg of adenosine and esmolol, each. Such an injection results in severe bradycardia and hypotension that decrease the MAP to 10–20 mm Hg with a precipitous decline in end-tidal CO₂ concentrations and cerebral blood flow (Joshi et al., 2006).

Physiological parameters spontaneously recover within the next 2–5 min without any inotropic support. The CHP challenge is reproducible and can be repeated safely within 30 min for at least 3 cycles.

4.2. NADH monitoring technique

Previously, NADH fluorescence has been assessed in rabbits using intra-vital microscopy (Tomlinson et al., 1993a, 1993b) where the dura was exposed and a large area of the brain was imaged during the experiment. In this study, we took a different approach for measuring the NADH redox state. It is based on the measurement of absorption and then emission of ultraviolet light only by NADH. Under constant ultraviolet light exposure the amount of ultraviolet light (366 nm) reflected back from the tissue, is a function of few factors, mainly the blood volume in the monitored site (Mayevsky and Rogatsky, 2007). The amount of fluorescent light emitted is a function of NADH redox state. Therefore during tissue hypoxia the sum of NAD and NADH will be unchanged and hence the fluorescence of NADH (450 nm) signal will increase. Thus, the difference between fluorescent (450 nm) and reflected ultraviolet (366 nm) light signals provides a measure of the relative change in NADH levels (Mayevsky and Rogatsky, 2007; Mayevsky et al., 2004).

In preliminary experiments using hypoxic challenge, we first determined that brain tissue NADH redox state could be measured through the dura in rabbit models. Measurements of NADH levels with our device in other animal species are feasible with craniotomy as small as 2 mm that permits placement of the fiber-optic probe. However, for this study we undertook a larger parietal craniotomy (5–6 mm diameter) so as to identify a relatively avascular area (with only small vessels) of the brain. The NADH probe was put in gentle contact with the brain using a micromanipulator. Fig. 1 provides a schematic illustration of our equipment. We use 366 nm ultraviolet light to excite NADH molecules and measure the resulting fluorescent light at 450 nm. The reflected and fluorescent light signals are converted into voltage output that can be recorded in real-time. The device is internally

calibrated using a 500 mV test signal and it yields relative changes in NAD/NADH concentrations after the baseline value is set to 0. A recent review by Mayevsky et al. describes the method in detail (Mayevsky and Rogatsky, 2007).

4.3. Treatment protocol

In our rabbit model we had a baseline propofol infusion rate of 200–400 µg/kg/min. To minimize the number of repeat challenges and to test the preparation, all animals were first subjected to transient global cerebral hypoperfusion, with background propofol anesthesia and an active EEG. Thereafter they were randomized to intraarterial or intravenous propofol pretreatment before the hypoperfusion challenge. With background propofol concentration, a bolus intravenous injection of 10–20 mg and an intraarterial injection of 3–5 mg propofol resulted in transient EEG silence lasting a mean duration of 2 min (Wang et al., 2003). For each challenge the data was collected for 10 min and then the preparation was allowed to recover for 30 min before the next challenge.

4.4. Data analysis

The data were collected at four time points for each challenge: baseline, during cerebral hypoperfusion, and 5 and 10 min post cerebral hypoperfusion. Factorial measures ANOVA will be used to analyze data within and then across the groups. Bonferroni–Dunn post-hoc test will be used for multiple comparisons. A *P* value of <0.0083 and <0.0167 for four and three repeat measures respectively will be considered significant.

Abbreviations

NADH	nicotinamide adenine dinucleotide hydrate
CBF	cerebral blood flow
NAD	nicotinamide adenine dinucleotide

References

- Avi-Dor Y, Olson JM, Doherty MD, Kaplan NO. Fluorescence of pyridine nucleotides in mitochondria. *J Biol Chem.* 1962; 237:2377–2383.
- Joshi S, Wang M, Hartl R. Retinal discoloration test. *J Cereb Blood Flow Metab.* 2004; 24:305–308. [PubMed: 15091111]
- Joshi S, Wang M, Etu JJ, Nishanian EV, Pile-Spellman J. Cerebral blood flow affects dose requirements of intracarotid propofol for electrocerebral silence. *Anesthesiology.* 2006; 104:290–298. (discussion 5A). [PubMed: 16436848]
- Joshi S, Meyers PM, Ornstein E. Intracarotid delivery of drugs: the potential and the pitfalls. *Anesthesiology.* 2008; 109:543–564. [PubMed: 18719453]
- Kasischke KA, Vishwasrao HD, Fisher PJ, Zipfel WR, Webb WW. Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science.* 2004; 305:99–103. [PubMed: 15232110]
- Mayevsky A, Rogatsky GG. Mitochondrial function in vivo evaluated by NADH fluorescence: from animal models to human studies. *Am J Physiol Cell Physiol.* 2007; 292:C615–C640. [PubMed: 16943239]

- Mayevsky A, Manor T, Pevzner E, Deutsch A, Etziony R, Dekel N, Jaronkin A. Tissue spectroscope: a novel in vivo approach to real time monitoring of tissue vitality. *J Biomed Opt.* 2004; 9:1028–1045. [PubMed: 15447025]
- Paschen W, Mies G, Hossmann KA. Threshold relationship between cerebral blood flow, glucose utilization, and energy metabolites during development of stroke in gerbils. *Exp Neurol.* 1992 Sep; 117 (3):325–333. [PubMed: 1397169]
- Pittman JE, Sheng H, Pearlstein R, Brinkhous A, Dexter F, Warner DS. Comparison of the effects of propofol and pentobarbital on neurologic outcome and cerebral infarct size after temporary focal ischemia in the rat. *Anesthesiology.* 1997; 87 (5):1139–1144. [PubMed: 9366466]
- Rampil IJ, Litt L, Mayevsky A. Correlated, simultaneous, multiple-wavelength optical monitoring in vivo of localized cerebrocortical NADH and brain microvessel hemoglobin oxygen saturation. *J Clin Monit.* 1992; 8:216–225. [PubMed: 1494928]
- Schmid-Elsaesser R, Schroder M, Zausinger S, Hungerhuber E, Baethmann A, Reulen HJ. EEG burst suppression is not necessary for maximum barbiturate protection in transient focal cerebral ischemia in the rat. *J Neurol Sci.* 1999; 162 (1):14–19. [PubMed: 10064163]
- Stryer, L. *Biochemistry*. Vol. Chapter 17. W.H. Freeman and Company; New York: 1988. Oxidative phosphorylation; p. 397–426.
- Tomlinson FH, Anderson RE, Meyer FB. Acidic foci within the ischemic penumbra of the New Zealand white rabbit. *Stroke.* 1993a; 24:2030–2039. [PubMed: 8248985]
- Tomlinson FH, Anderson RE, Meyer FB. Brain pHi, cerebral blood flow, and NADH fluorescence during severe incomplete global ischemia in rabbits. *Stroke.* 1993b; 24:435–443. [PubMed: 8446980]
- Wang M, Joshi S, Emerson RG. Comparison of intracarotid and intravenous propofol for electrocerebral silence in rabbits. *Anesthesiology.* 2003; 99:904–910. [PubMed: 14508324]
- Warner DS, Takaoka S, Wu B, Ludwig PS, Pearlstein RD, Brinkhous AD, Dexter F. Electroencephalographic burst suppression is not required to elicit maximal neuroprotection from pentobarbital in a rat model of focal cerebral ischemia. *Anesthesiology.* 1996; 84 (6):1475–1484. [PubMed: 8669689]

Fig. 1.

The schematic representation of the ultraviolet spectroscopy device that uses light at 366 nm to excite the NADH molecules. NADH fluoresces at the 420–480 nm range. This device measures reflectance at 450 nm.

Fig. 2.

Changes in physiological parameters at four stages [baseline, cerebral hypoperfusion (CHP), 5 min post CHP and 10 min post CHP] during all three challenges (Control, IV-propofol and IC-propofol). CHP stage is significantly different from other stages of the experiment for all three challenges (denoted by an asterisk).

Fig. 3.

Changes in NADH at four stages [baseline, cerebral hypoperfusion (CHP), 5 min post CHP and 10 min post CHP] during the three challenges (Control, IV-propofol and IC-propofol). CHP stage is significantly different from other stages of the experiment for all three challenges (denoted by an asterisk).

Fig. 4.

For a given animal, the figure shows a relative change in NADH in relation to (a) the mean arterial pressure and (b) %-change in cerebral blood flow.

Fig. 5.

The proposed relationship between the relative changes in NADH and cerebral blood flow during transient cerebral ischemia creates a hysteresis loop (interrupted line). Neuroprotection by anesthetic drugs, by suppressing synaptic transmission, should have changed the shape of the curve and shifted to the left (solid line). Experimental data shown in Fig. 4b does show a left shift but there is no attenuation of NADH increase during transient cerebral hypoperfusion.

Fig. 6.

The graph illustrates the effect of propofol induced EEG silence on cerebral blood flow, mean arterial pressure and NADH levels with or without phenylephrine. Administration of phenylephrine to maintain mean arterial pressure in the normal range leads to only a minimal (7–10%) rise in NADH in the absence of phenylephrine.

Table 1

Changes in physiological parameters during transient cerebral hypoperfusion.

	Treatment	Baseline	CHP	5 min post CHP	10 min post CHP
Esophageal temperature (°C)	Control	36.6±0.9	36.4±0.9	36.2±1.0	36.4±1.0
	IC-propofol	36.6±0.9	36.4±0.9	36.6±1.1	37.1±1.0
	IV-propofol	36.2±1.0	36.1±1.0	36.0±0.9	36.8±1.0
Brain temperature (°C)	Control	34.9±1.5	34.5±1.8	34.6±1.8	34.7±1.8
	IC-propofol	34.8±1.8	34.5±1.8	34.8±1.9	35.0±1.3
	IV-propofol	34.0±2.0	34.2±1.6	34.2±1.9	35.0±1.8
ETCO ₂ (mm Hg)	Control	43±9	19±6 ^a	40±14	43±9
	IC-propofol	42±8	17±8 ^a	43±8	42±8
	IV-propofol	41±8	23±14 ^a	42±8	42±8
Respiration rate (/min)	Control	65±9	65±9	65±9	65±9
	IC-propofol	65±9	65±9	65±9	65±9
	IV-propofol	65±9	65±9	65±9	65±9
Heart rate (beat/min)	Control	250±25	105±49 ^a	232±25	254±28
	IC-propofol	257±24	111±58 ^a	239±31	257±24
	IV-propofol	250±24	82±58 ^a	241±33	262±30
LDI (%change)	Control	100±0	10±6 ^a	149±32 ^b	90±24
	IC-propofol	100±0	12±6 ^a	164±49 ^b	106±15
	IV-propofol	100±0	11±3 ^a	144±49 ^b	103±19
LDC (%change)	Control	100±0	9±6 ^a	177±40 ^b	97±28
	IC-propofol	100±0	10±4 ^a	176±53 ^b	119±22
	IV-propofol	100±0	10±5 ^a	162±32 ^b	107±32
NADH	Control	0	0.29±0.13	-0.01±0.06	0.01±0.06
	IC-propofol	0	0.35±0.13	-0.01±0.06	0.01±0.05
	IV-propofol	0	0.35±0.11	0.021±0.05	0.01±0.04
MAP (mm Hg)	Control	98±11	18±5 ^a	81±12	89±10
	IC-propofol	98±16	18±4 ^a	87±14	96±18

Treatment	Baseline	CHP	5 min post CHP	10 min post CHP
IV-propofol	89±10	17±4 ^a	75±23 ^c	94±16

Data presented as mean±standard deviation. CHP: cerebral hypoperfusion; ET/CO₂: end-tidal carbon dioxide tension; LDI: laser Doppler flow-ipsilateral; LDC: laser Doppler flow-contralateral. MAP: mean arterial pressure.

^a CHP stage significantly different from other stages of the experiment.

^b 5 min post-hypoperfusion significantly different from other stages of the experiment.

^c 5 min post-hypoperfusion significantly different from 10 min post-hypoperfusion.