Neuroscience Letters 541 (2013) 58–62

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Dose-dependent effect of isoflurane on regional cerebral blood flow in anesthetized macaque monkeys

Chun-Xia Li[a], Sudeep Patel[a], Edward J. Auerbach[b], Xiaodong Zhang[a,c,*]

[a] Yerkes Imaging Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, United States
[b] Center for MR Research, School of Medicine, University of Minnesota, Minneapolis, MN 55455, United States
[c] Division of Neuropharmacology and Neurologic Diseases, Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, United States

HIGHLIGHTS

- Regional CBF effects are poorly understood in monkeys under maintenance doses of isoflurane.
- Isoflurane results in global CBF increase in high isoflurane dose level.
- CBF is altered significantly in subcortical structures in the dose range 0.75–1.5%.
- CBF auto-regulation is preserved in cortical regions but disturbed in thalamus and cerebellum.

ARTICLE INFO

Article history:
Received 3 October 2012
Received in revised form 31 January 2013
Accepted 6 February 2013

Keywords:
Dose-dependent effect
Isoflurane
Cerebral blood flow (CBF)
Auto-regulation
Non-human primate
Arterial spin labeling (ASL)

ABSTRACT

The dose-dependent effect of isoflurane on regional CBF of cortical and subcortical structures in anesthetized macaque monkeys was investigated with the Continuous ASL MRI technique. High concentration of isoflurane resulted in global CBF increase and blood pressure decrease. Evident CBF change was observed in the subcortical structures. Specifically, CBF in thalamus and cerebellum was increased about 39% and 55% when isoflurane concentration was changed from 0.75% to 1.5%, respectively. Also, those regional CBF changes correlated linearly with isoflurane inspiratory concentrations, indicating impaired CBF autoregulation in these structures. In contrast, no obvious CBF changes were observed in anterior cingulated cortex, motor cortex, medial prefrontal cortex, and caudate. The results demonstrate that, under the 0.75–1.5% isoflurane maintenance doses, the CBF auto-regulation is well preserved in the cerebral cortical regions and caudate, but impaired in thalamus and cerebellum, indicating disturbed CBF-metabolism coupling and functional response in specific subcortical regions of anesthetized macaque monkeys.

1. Introduction

Isoflurane is a volatile inhalation anesthetic agent and commonly used in surgical procedures and in vivo neuroimaging examinations of animals and humans. Previous human and animal studies indicate that the induction of isoflurane results in cerebral vasodilatation and has evident effects on cerebral blood flow (CBF), cerebral blood volume (CBV), permeability, neurovascular coupling, neuron functionality [1,5,7,8,11,12,15]. Increased CBF and reduced cerebral metabolic rate of oxygen consumption have been observed in the animal and human studies [1,11]. Also, previous canine, baboon, and human studies have shown that the CBF autoregulation mechanism was disturbed under high dose isoflurane [9,11,20].

Non-human primates (NHPs) resemble humans more than any other animals and are widely used in preclinical studies and various neuroscience researches. As isoflurane produces rapid induction and recovery from anesthesia, most NHP examinations and neuroimaging experiments (such as MRI and PET) are performed under isoflurane anesthesia. The 0.75–1.5% isoflurane, mixed with 100% O_2 or ambient air, is normally used as the maintenance dose for sedation purpose during general structural MRI, functional MRI or PET scanning. In each scanning session, the isoflurane concentration may be adjusted to obtain light or deep depth of anesthesia for specific study purpose or to accommodate to each subject's physiological or pathological condition. Evident effects of large isoflurane dose variation on CBF have been reported in animal and human studies [9,11,20]. BOLD functional MRI responses in the default-mode network of the anaesthetized monkey brain (under 0.8–1.5% isoflurane) have demonstrated evident reduction of neural activation under deep anesthesia (light vs deep anesthesia) [21]. A

* Corresponding author at: Yerkes Imaging Center, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road NE, Atlanta, GA 30329, United States. Tel.: +1 404 712 9874; fax: +1 404 712 9807.
E-mail address: xzhang8@emory.edu (X. Zhang).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.02.007

prior Xenon-133 study of anesthetized baboons has showed that isoflurane produces vasoconstriction in cerebral vessels in the low doses (~0.5% or less)) or vasodilation in the high doses (~0.95% or more), indicating there exist the transition doses of the biphasic CBF responses between the low and high doses [20]. The 0.75–1.5% maintenance doses are within the range of the transition doses of the biphasic characteristic. However, due to the technique limitation in high-resolution CBF measurements in prior studies, the relevant dose-dependent effect of isoflurane on regional CBF in different cortical and subcortical structures is poorly understood.

The arterial-spin-labeling (ASL) MRI perfusion technique provides a unique means to quantitatively measure high-resolution CBF of humans and animals non-invasively [2,16,18]. In comparison with other perfusion techniques, ASL offers higher spatial resolution and temporal resolution to measure the brain hemodynamic response and has been used widely to access the neuronal activation and acquire basal CBF maps under various circumstances. As the continuous ASL(CASL) technique with a separate labeling coil provides improved signal-to-noise ratio(SNR) and reduced RF exposure, a specific setting with the CASL technique has been successfully developed to measure whole brain CBF of macaque monkeys at high-resolution and SNR [22]. In the present study, the specific CASL technique was employed to investigate the dose-dependent effect of isoflurane on regional CBF in the cortical and subcortical structures of anesthetized NHPs.

2. Methods and materials

2.1. Animal preparation

Adult healthy female rhesus monkeys ($n = 4$, 6–10 years old) were used in this study. The animals were initially anesthetized with ketamine (5–10 mg/kg, IM), then orally intubated. An IV catheter was placed for delivering lactated ringers solution (3.5–10 ml/kg/h) in the scanner. The anesthetized and spontaneously breathing animals were immobilized with a custom-made head holder and placed in the "supine" position during MRI scanning. Isoflurane was administrated at three inspiratory concentrations with random order: 0.75%, 1.0%, and 1.5% (0.6, 0.8. 1.2 MAC (minimum alveolar concentration) (end-tidal), respectively), mixed with ambient air. Et-CO_2, inhaled CO_2, O_2 saturation, blood pressure, mean arterial pressure (MAP), heart rate, respiration rate, and body temperature were monitored continuously in addition to visual inspection of animals every 30 min. These physiological parameters were recorded and maintained in normal ranges (PCO_2: 38–42 mmHg; PO_2: 25–35 mmHg; O_2 saturation: 95–100%; MAP: 60–100 mmHg; heart rate: 120–150 beats/min) in each study session. The body temperature was maintained at 37.5 °C by a feedback-regulated circulating warm-water blanket. All procedures followed the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Emory University in accordance with the NIH Guide for Care and Use of Laboratory Animals.

2.2. MRI examination

MRI scans were performed on a Siemens 3T Trio whole body scanner (Siemens Medical, PA, USA) with an 8-channel high resolution knee coil (Invivo, Inc.) and a home-made butterfly neck coil (ID = 2.9 cm, each loop) with active decoupling [22]. Isofluance concentrations were monitored and measured continuously with an anesthesia monitor (GE Datex – ohmeda Cardiocap/5). A cross-sectional image of the neck was taken to verify that the neck labeling coil was properly positioned. The single-shot, partial-Fourier, gradient-echo planar imaging (EPI) was applied for the CBF measurement. The MRI parameters were: TR/TE = 4000/25 ms, FOV = 96 mm × 96 mm, data matrix = 64 × 64, 16 slices with slice thickness = 1.5 mm, post-labeling-delay = 0.8 s, Labeling duration= 2 s. Seventy pairs of control and labeling axial images were acquired and the acquisition was repeated 3 times at each dose. Corresponding T_2 weighted images also were acquired by using fast spin-echo sequences with TR/TE = 5900/125 ms, FOV = 96 mm × 96 mm, matrix = 128 × 128, 2 averages. The CBF measurement was started at least 30 min later after the animal was moved into the scanner. The 15-min minimum equilibrium time was applied after isoflurane dose was changed each time.

2.3. Data analysis

Data analyses were performed using Matlab (MathWorks, MA) and Stimulate software (http://www.cmrr.umn.edu/stimulate). CBF maps were calculated with the formula and parameters in our previous paper [22] except that a constant T_1 (1.0 s) of brain tissue (gray matter and white matter) was applied instead of a measured T_1 map. Also, the equilibrium value M_0 was approximated with the amplitude of the correspondent non-tagged image, as the relative CBF changes were required in the present study and evaluated in data analysis. The simplified formula is

$$\mathrm{CBF} = \frac{\lambda}{2a} \times \left(1 - \frac{S_{\text{labeled}}}{S_{\text{non-labeled}}}\right) \times \frac{1}{e^{-\frac{\mathrm{ATT}}{T_{1A}}} \times e^{-(\mathrm{PLD}-\mathrm{ATT})} \times (1 - e^{-\mathrm{LD}})}$$

where λ is the water brain–blood partition coefficient, α is the arterial spin-labeling efficiency, $S_{\text{non-labeled}}$ and S_{labeled} are signal intensities of the non-labeled and labeled images, respectively, ATT is the arterial transit time, T_{1A} is the longitudinal relaxation time (T_1) of the arterial blood at 3T, PLD is the post-labeling delay and LD is the labeling duration. All the experiment parameters were as same as described in our previous paper. Briefly, $\alpha = 0.92$, $\lambda = 0.98$ ml/g for gray matter and 0.82 ml/g for white matter, ATT = 0.8 s, $T_{1A} = 1.66$ s, LD = 2 s, PLD = 0.8 s. PLD for different imaging slices were adjusted in the CBF calculation based on known acquisition time per slice.

The motor cortex, medial frontal cortex (mPFC), anterior cingulated cortex (ACC), caudate, thalamus, cerebellum, were selected for region of interest (ROI) analysis (Fig. 1). Each ROI was outlined based upon the corresponding non-tagged high resolution EPI images and T_2-weighted images with the Stimulate software. For each animal, CBF of each ROI was normalized to the mean CBF value of the three dose levels to reduce the inter-subject variations of CBF measurements in the data analysis. Repeated ANOVA was performed to analyze the CBF differences statistically across the different doses. Correlation analysis was used to study the dose-dependence effects on CBF. SPSS 17.0 was used for statistical analysis. P-values less than 0.05 were considered statistically significant.

3. Results

CBF in each monkey was obtained under 0.75%, 1.0%, and 1.5% isoflurane. The isoflurane dose-dependent effects on global, cortical and subcortical mean CBF are shown in Fig. 2. CBF in global and subcortical regions were increased 14% (±5%) and 25% (±4%) (1.5% vs 0.75% isoflurane), respectively. The corresponding CBF changes in different brain structures are illustrated in Fig. 3. Specifically, regional CBF changed about 4.5% (±1.9%) in frontal cortex, ACC and motor cortex, and 10% (±5%) in caudate. There was no significant difference observed between the high dose groups and the low dose base line. However, CBF in thalamus and cerebellum were increased 39% (±23%) and 55% (±15%) (1.5% vs 0.75% isoflurane), respectively. In addition, the changes were linearly and significantly correlated