Systemic Effects Of Anesthesia On The fMRI-BOLD Signal Response During Apnea In Rats

S. S. Kannurpatti[1], B. B. Biswal[1]

[1]Radiology, UMDNJ-New Jersey Medical School, Newark, NJ, United States

Introduction: In the present study systemic effects of anesthesia on the dynamics of the apnea-induced Blood Oxygen Level Dependent (BOLD) signal was investigated. Cerebral oxygenation and blood flow dynamics was studied in rats in response to apnea using fMRI and laser doppler flowmetry (LDF) respectively. From earlier studies it is known that urethane anesthesia does not influence GABAergic mechanisms involved in the control of cardiovascular functions unlike pentobarbital (1,2). We hypothesized that pentobarbital and urethane would have distinct systemic effects and their influence on the MAP, CBF and the fMRI-BOLD signal dynamics was investigated.

Methods: Male Sprague Dawley rats 250-300 g (n=20) was used for all studies and separated into four groups. The first two groups for fMRI and the last two groups for LDF studies. The first and third groups (n=5 each) were anesthetized with sodium pentobarbital (60mg/Kg i.p). If an increase in the mean arterial pressure above 10% of the normal level was observed in response to a tail pinch, a supplementary dose of 15mg/Kg i.p was administered. The second and fourth groups were anesthetized with urethane (1.2 g/Kg i.p) and supplemented with 20% of the initial dose subsequently if blood pressure increased to a tail pinch during the protocol. Body temperature was monitored with a rectal probe and maintained at 37.0±0.5 ^{o}C using a homeothermic feedback heating system (Baxter K-MOD100, Gaymar Industries). The animals were endotracheally intubated and mechanically ventilated with air. Gallamine triethiodide (250mg/Kg i.p), a paralyzing agent was administered at the start of ventilation to prevent spontaneous breathing of the rats. The femoral arteries were cannulated with PE50 tubing for mean arterial blood pressure (MAP) measurements and blood gas sampling. Arterial blood pressure, end-tidal CO_2 and inspired oxygen concentration were continuously monitored (POET II, Criticare Systems). In rat groups used for LDF studies, the head was secured to a stereotaxic frame using ear bars and the scalp was retracted from the frontoparietal cortex by a midline incision. The temporalis muscle was disconnected from the skull. The skull area of 5x5 mm enclosing the sensorimotor cortex was thinned to translucency on either hemisphere using a dental drill. The underlying vasculature was visible when washed with saline. Laser Doppler signals were measured after placing the probe over the thinned skull window immersed in mineral oil.

Functional MRI: fMRI experiments were carried out using a Bruker Medspec 3T/60cm imaging system. A custom-built 5.0 cm long and 3.8 cm diameter quadrature RF bird-cage coil was used to obtain images in the rat. To minimize motion artifacts, the rat was secured to the RF coil by a bite bar resting below the upper hard palate and over the snout. Coronal localization of slices was accomplished using an initially obtained mid-line sagittal slice and comparing with the sagittal section from a rat brain atlas. Three contiguous coronal slices of thickness 2 mm were selected covering the region 0 to -6mm from the Bregma. Anatomical images were obtained before fMRI scanning using a RARE sequence with TR=1sec, TE=19msec, 256x256 matrix and FOV=3.5 cm. A single shot gradient EPI sequence was used for functional imaging using a 64x64 matrix, TR/TE=2sec/27.2msec, slice thickness=2mm and a bandwidth of 125 kHz. One hundred and sixty five images were obtained for each slice in a dynamic scanning time of 5 min. The first scan consisted of acquisition of 165 images during rest followed by the second scan in which apnea was administered. Images were collected for 60 sec during normal breathing alternating with 20 sec of apnea in three epochs. Apnea was induced by switching off the ventilator. These experiments were repeated with the rat ventilated sequentially with various gas mixtures namely room air, 100% O_2, carbogen, 2% CO_2 and 5% CO_2 respectively.

Laser -Doppler Flowmetry: On the third and fourth rat groups, CBF measurements were carried out using a laser Doppler flowmeter and a 0.5 mm needle probe (Oxford Array™, Oxford-Optronix Ltd., Oxford). The probe was placed on the thinned skull and positioned over the sensorimotor cortex area using a micromanipulator. Continuous monitoring of LDF was possible with a spatial resolution of 1 mm^3 and a temporal resolution of 0.2 sec. The experimental paradigm was similar to the fMRI studies.

Results: FMRI-BOLD signals were measured in two rat groups in response to 20 sec of apnea while anesthetized with pentobarbital or urethane. Apnea induced a global change in the fMRI-BOLD signal. In rats ventilated with room air, BOLD signal intensity decreased at the onset of apnea. Fig. 1a and b show typical time courses of the BOLD signal intensity from the sensorimotor cortex, in rats ventilated with room air and anesthetized with sodium pentobarbital or urethane respectively. The typical onset time (t_0) of the decrease in BOLD signal intensity after induction of apnea was 3-4 sec. No significant differences were observed in the onset time for signal decrease (t_0) between the two anesthetics. However the time for maximum signal decrease, (t_{max}) averaged over all rats was 17±1 sec with pentobarbital anesthesia, which was significantly higher than 15±1 sec ($P<0.05$) when rats were anesthetized with urethane. Upon resumption of ventilation, the BOLD signal recovered with an immediate increase followed by a gradual rise to a pre-apnea baseline level in the next few minutes. With room air ventilation, the average decrease in BOLD signal intensity in the brain region covered by the images for all rats was 7.2±1.4% with pentobarbital and 9.0±3.9% with urethane anesthesia respectively. To quantify the changes in CBF, LDF signals were measured from the sensorimotor cortex region in a different group of rats anesthetized with pentobarbital or urethane.

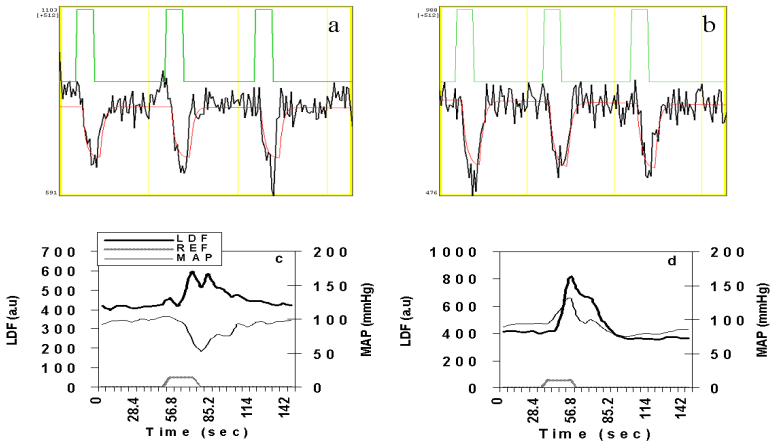

Fig 1. Time courses of the BOLD signal intensity (black) boxcar reference (green) and the corresponding non-linear fit (red) from a representative voxel in the sensorimotor cortex. Rats were ventilated with room air and anesthetized with, **a)** pentobarbital and **b)** urethane. Traces of LDF and MAP signals during apnea from a typical rat ventilated with room air and anesthetized with **c)** pentobarbital and **d)** urethane.

Fig 1c shows traces of typical LDF and MAP signals from a rat ventilated with room air and anesthetized with pentobarbital. Apnea induced a 43.6±8.35% increase in LDF in rats anesthetized with pentobarbital under normoxic conditions. There was a parallel drop in MAP with a peak decrease of 17.1±5.9% during this duration. The arterial oxygen saturation decreased from 97% to 28% at the end of 20 sec apnea. Fig 1d shows typical LDF and MAP signals from a rat anesthetized with urethane and ventilated with room air. In urethane-anesthetized rats ventilated with room air, apnea-induced increase in LDF was 101.5±14.9% during which there was a parallel increase in MAP by 31.3 ±14.2%. The arterial oxygen saturation decreased from 96% to 42% at the end of 20 sec apnea.

Conclusions: In conclusion, the amplitude of the apnea-induced BOLD signal response in anesthetized rats is independent of changes in MAP or CBF during normoxic conditions. Pentobarbital anesthesia, which affects GABAergic mechanisms, has a significantly different effect with respect to BOLD signal onset time, percent changes in CBF and MAP when compared to urethane anesthesia, which does not affect GABAergic mechanisms. Anesthesia-dependent MAP change modulates the apnea-induced CBF response but has a minimal effect on the fMRI-BOLD signal probably due to uncoupling of CBF and oxygen consumption.

References

1. Maggi CA, Meli A. Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Experientia, 1986; 42:109-114.
2. Yamada KA, Moerschbaecher JM, Hamosh P, Gillis RA. Pentobarbital causes cardiorespiratory depression by interacting with a GABAergic system at the ventral surface of the medulla. J Pharmacol Exp Ther 1983; 226: 349-355.