PhMRI of Brain Deactivation: Effects of the Antiepileptic Agent Tiagabine on Cerebral Haemodynamics

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Introduction

While the fMRI response to excitatory somatosensory and pharmacological stimuli is well characterised, negative responses are more difficult to interpret and have been the subject of much debate in the recent literature [1]. Here, we have employed a multimodal approach to investigate the individual components of the haemodynamic response to pharmacological challenge with Tiagabine, a GABAergic drug with anticonvulsant properties [2], in the anaesthetised rat. Tiagabine inhibits reuptake of GABA, the principal inhibitory neurotransmitter in the brain, thus inducing widespread cortical deactivation [3]. We have used MR methods, Laser Doppler Flowmetry, and fluorescence quenching methods to measure the changes induced by Tiagabine in cerebral blood volume, blood flow and tissue oxygen tension, respectively. Oxygen levels in the brain parenchyma are sensitive to the balance between oxygen supply and consumption, and provide important information to correlate haemodynamic changes with underlying changes in brain metabolism.

Methods:

All experiments were carried out in accordance with Italian regulations governing animal welfare and protection. Protocols were also reviewed and consented to by a local animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985).

LDF and pO2 measurements: Male Sprague-Dawley rats (250-350g) were anaesthetised with 3% halothane in O_2:N_2 1:2, tracheotomised and mechanically ventilated under infusion of the neuromuscular blocker D-tubocurarine. The femoral artery was cannulated to monitor arterial blood pressure and blood gas levels. Student t test analysis of blood gases data did not evidence systematic differences between pCO2 or pO2 values pre- and post-MRI, or between groups. Subsequently, the animals were placed in a stereotaxic frame and implanted with a combined fluorescence-quenching/Laser-Doppler-Flow (LDF) probe (Oxylite, Optronix) in the motor cortex probe (M1, n=8; AP +2.2 mm, ML +2.8 mm, DV -2.5 mm). After surgery, the anaesthesia was decreased to 1% halothane for maintenance. After 2h stabilisation, animals were challenged with tiagabine (10 mg/kg i.v., n=8) or its vehicle (β-hydroxy-propyl-cyclodextrin, 0.05 g/ml in saline, n=8). The arterial blood pressure response to tiagabine was modest (+ 17%) and not significantly different from the vehicle.

rCBV measurements: MRI experiments were performed on male Sprague-Dawley rats, prepared as described elsewhere [4;5]. The data were acquired using a Bruker Biospec 4.7T system, a 72mm birdcage resonator for RF transmit and a quadrature surface receive coil (Bruker, Ettlingen, Germany). The time series data were acquired using the RARE sequence: matrix 128x128; FOV 40mm; slice thickness 2mm; 8 contiguous coronal slices; RARE factor 32; TE_{eff}=110ms; TR=2700ms; δt=40s. A 2.67 ml/kg dose of Endorem blood pool contrast agent (Guerbet, France) was administered i.v. following 5 reference image frames, to sensitise the acquisition to changes in CBV as described in [6]. After 20 min stabilisation period, animals were challenged with tiagabine (10 mg/kg i.v., n=5) or its vehicle (β-hydroxy-propyl-cyclodextrin, 0.05 g/ml in saline, n=5).

Results and conclusions:

Acute tiagabine challenge resulted in a significant reduction in cortical rCBV (-12.9±2.5%, p<0.01 vs. vehicle). The effect peaked 3 minutes after injection and was followed by a slow return to pre-injection baseline values within 20 minutes. The rCBV response to tiagabine was accompanied by a marked (-25%) and sustained (> 25 min) reduction in LDF (p<0.05 vs. vehicle). This was paralleled by a sustained (> 25 min) increase in tissue pO2 (+18 %, p<0.05). Intravenous administration of vehicle produced transient increase in pO2 which was temporally correlated with a short-lived increase in MABP induced by the IV injection.

Overall, the tiagabine-induced changes in LDF and pO2 showed a negative temporal correlation, with a very similar time-profile, characterized by a stable and persistent effect over the whole time window of this study. Interestingly, a reduced tissue perfusion was accompanied by increased tissue oxygen tension, thus suggesting that during cortical GABAergic deactivation, tissue oxygen consumption decreases more than oxygen supply.

It should be noted that increases in tissue oxygen levels are also observed with excitatory challenges like cocaine, which induces positive CBV and CBF changes [7,8]. Therefore, CBF and CBV measurements appear to be more unequivocal indicators of the excitatory or inhibitory effects of the drugs than oxygen levels.

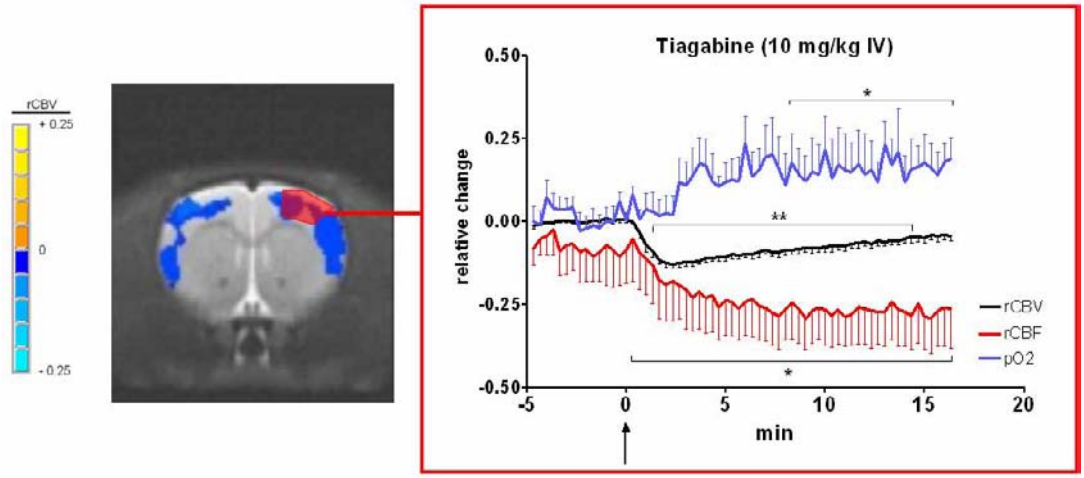

Figure 1. Left**:** Map of the significant rCBV decrease induced by tiagabine. Blue areas indicate *decreased* rCBV vs. vehicle (p<0.05). The red circle indicates the anatomical location of the motor cortex area used for the temporal analysis of the haemodynamic data. Right: Temporal profile of rCBV, rCBF and tissue pO2, in the motor cortex following IV administration of tiagabine. (* p<0.05 vs. vehicle; **p< 0.01 vs. vehicle).

References

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