The selective mGluR5 receptor antagonist MPEP attenuates fMRI response to forepaw stimulation in the rat: Effects on brain oxygen pressure, cerebral blood flow and volume.

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Introduction

Several functional neuroimaging methods, including fMRI, measure the haemodynamic response as surrogate readout for neuronal activity [1]. However, the physiological mechanisms underlying the coupling between neuronal activity, brain metabolism and vascular reactivity are still under investigation. Recently, Zonta and co-workers [2] demonstrated the role of metabotropic glutamate receptors (mGluR5 and 1) in the control of vascular reactivity through [Ca2+] increase in astrocytes' soma. These authors reported that blockade of mGluR5 and mGluR1 receptors leads to a decrease of the calcium influx in the astrocyte evoked by direct neuronal stimulation, and to a decrease of arteriole dilation. The aim of the present study was to investigate the effects of blockade of mGluR5 receptors on the vascular response evoked by forepaw stimulation in the rat, and the resulting changes in brain partial oxygen pressure.

Methods

Experiments are carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), and Italian legislation regarding animal use (DL 116/1992). Experiments were conducted in two separate groups. In the first one, 7 male Sprague-Dawley rats (250-350g) were used. Anaesthesia was induced by Isoflurane (5%) and maintained at 2-3% during surgery. Animals were mechanically ventilated under neuromuscular blocker (d-Tubocurarine 0.25 mg/kg in bolus followed by a continuous infusion at 0.25 mg/kg/hr). Anaesthesia was then switched to α-chloralose (bolus i.v. of 50 mg/kg followed by a continuous infusion at 40 mg/kg/hr). Cerebral activation was induced through electrical stimulation (2Hz, 2mA, 0.3ms) using subdermal electrodes placed over the wrist. Two hours after the beginning of chloralose anaesthesia, brain oxygen partial pressure (pO_2) and cerebral blood flow (CBF) were measured simultaneously using the local probe Oxylite (Oxford Optronix) inserted into layer IV of the contralateral somatosensory cortex. Repeated stimulation of the forepaw was applied (3min OFF-45s ON). MPEP (0.5 mg/kg i.v.) was injected after 5 stimulation blocks.

The same animal preparation protocol was used in the second group (n = 5, 250-350g) in which we acquired MRI data using a Bruker Biospec 4.7T system, a 72mm birdcage resonator for RF transmit and a quadrature surface receive coil (Bruker, Ettlingen, Germany). Cerebral blood volume (CBV) changes were assessed using the RARE sequence [3]: matrix 128x128; FOV 40mm; slice thickness 2mm on one slice centred at 0.2 mm rostral to the Bregma, TE_{eff}=110ms; TR=2500ms. A 2.67 ml/kg dose of Endorem blood pool contrast agent (Guerbet, France) was administered i.v. following 3 stimulation blocks (3 min OFF-60s ON). Prior to the CBV transformation [4], data were smoothed in the time direction using a 5 points Gaussian filter. The resulting data was then analysed using Stimulate [5] to produce statistical maps of brain activation (t-test, p=0.001).

Results

Fore-paw stimulation induced an increase in CBF, CBV and tissue pO_2 in the somatosensory cortex contralaterally, but not ipsilaterally to the electrical stimulation, consistent with previous reports. Injection of 0.5 mg/kg of MPEP (mGluR5 antagonists) resulted in a significant reduction of pO_2 and CBF increase (-41 ± 13 % * and -38 ± 7 % **respectively, means ± SEM * P<0.05, ** P<0.01 paired t-test) 45 min. after the injection (Fig. 1B,C). Analysis of the rCBV fMRI experiment showed a similar effect 40 min after the injection (-71 ± 7 % *, * P<0.05 paired t-test, Fig. 1A).

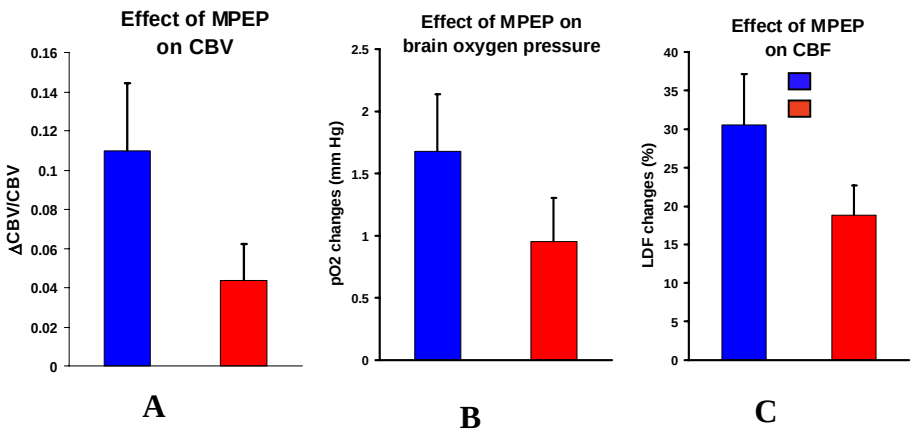

Fig 1: *Effect of MPEP (0.5 mg/kg) on cerebral blood flow (CBF), brain oxygen partial pressure (pO_2) and cerebral blood volume (CBV) evoked by forepaw stimulations. Histograms illustrate the effect of 0.5 mg/kg of MPEP on CBV (A), pO_2 (B) and CBF (C). Data are expressed as Means ± SEM. PO_2 and CBF data (N = 7) represents the average of 4 stimulation blocks before and 45 min. after injection of MPEP. CBV data (N = 5) represents the average of 3 stimulation blocks before and 45 min. after injection of MPEP. CBV regions of interest were obtained from statistical map of rCBV weighted activation (expressed as confidence) determined through a t-test on the three first stimulation blocks, with a confidence threshold of 0.999.*

Discussion

The main finding in this study is that a reduction of vascular response to forepaw stimulation (-38% for CBF and -71% for CBV) is accompanied by a reduction in brain tissue pO_2 change (-41 ± 13 %), indicating a lower evoked metabolic activity in the somatosensory cortex. As suggested by Zonta et al. [2], one might assume that MPEP is acting only on astrocyte activity by antagonising the mGluR5 receptor, as somatosensory evoked potentials are not affected using the concentration of MPEP used in this study. Hence, a reduced brain oxygen pressure is not necessarily the result of a reduction in neuronal metabolism, despite MPEP was found to act as a non-competitive antagonist at NMDA receptors [6].

References

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