Functional mapping of intracerebroventricular (ICV) infusion of neuroactive compounds in the anaesthetised rat

A. Gozzi[1], A. J. Schwarz[1], T. Reese[1], V. Crestan[2], S. Bertani[2], G. Turrini[2], M. A. Corsi[3], A. Bifone[1]

[1]Neuroimaging, GlaxoSmithKline Psychiatry Centre of Excellence for Drug Discovery, Verona, Italy, [2]Laboratory Animal Sciences, GlaxoSmithKline, Verona, Italy, [3]Neuropharmacology, GlaxoSmithKline Psychiatry Centre of Excellence for Drug Discovery, Verona, Italy

Introduction

Neuropeptides, such as Substance P, are implicated in numerous physiological functions and associated with many disease states. However, these compounds exhibit distinct peripheral and central effects that may be confounded when delivered systemically. The functional correlates of the central activity induced by these peptides remain to be elucidated. Intracerebroventricular (ICV) infusion is established procedure in behavioural and neurochemical studies of the central activity of such compounds [1]. In this study we have applied pharmacological MRI (phMRI) methods to map the haemodynamic response to ICV infusion of neuroactive compounds. Specifically, we have studied the phMRI response to central administration of bicuculline, a potent $GABA_A$ antagonist and of Substance P, a peptidic neurotransmitter implicated in schizophrenia, depression and anxiety [2].

Methods

MR-compatible guide cannulae (Bilaney consultants, Germany) were stereotactically implanted in to the right lateral ventricle of 10 male Sprague-Dawley rats. At least seven days of recovery were allowed before the phMRI study. Surgical preparation for the MR experiments included tracheotomy for artificial ventilation, femoral artery and vein cannulation for infusion of paralysing and contrast agents, and monitoring of blood gases. Anaesthesia was induced with 2.5% halothane, decreased to 1.5% for the surgical procedure, and then lowered to maintenance level of 0.8% for the MR experiment. MRI data were acquired using a Bruker Biospec 4.7T system. The time series experiment comprised 256 time points using the RARE sequence [3] matrix 128x128; FOV 40mm; slice thickness 2mm; 8 contiguous coronal slices; TE_{eff}=110ms; TR=2700ms; δt=10s. After the acquisition of a set of baseline images, the blood pool contrast agent Endorem (Guerbet, France) was administered i.v. (2.67 ml/kg) to sensitise the images to changes in Cerebral Blood Volume (CBV). Following a 20-35 min delay, either Substance-P (36, 100 or 300 nmol; n=7) or vehicle (saline; n=3) was manually infused ICV (5-9 µl in 60 s). In 9 animals, after the acquisition of the first set of data, the experiment was repeated with an additional ICV challenge of 26 nmol (5 µl) of bicuculline, as a positive control. In the image analysis, signal changes were expressed as percentage changes relative to pre-challenge baseline (conversion to Cerebral Blood Volume changes was not possible for the second challenge as the animal was moved between first and second injections).

Results and discussion

High-resolution anatomical images of cannulated animals highlighted visible brain asymmetry in the ipsilateral ventricles of 7 out of 10 animals. Asymmetric regions showed a bright, oedematous appearance, and extended throughout the lateral ventricle. These morphological abnormalities may be ascribed to implantation procedure. During ICV infusion a strong local increase in signal intensity in the ipsilateral cannulated ventricle occurred due to the injection of fresh fluid (Figure 1(a)). Subsequently, the ventricular signal intensity typically declined, reflecting the equilibration of the excess fluid with the CSF in the interconnected ventricles. ICV infusion of the $GABA_A$ antagonist bicuculline produced widespread and long-lasting activation in all the animals tested. The distribution and profile of bicuculline response were similar to that reported following peripheral administration [3]. The use of bicuculline, a potent and well-characterised brain stimulant, allowed us to exclude severe physiological impairments of the cerebro-vasculature (vascular reactivity) caused by the surgical implantation of the cannulae. Infusion of saline did not result in any significant signal change in brain tissue. ICV infusion of Substance P evidenced significant brain activation in 4 of the 7 animals studied (Figure 2(b)). The spatial distribution and the magnitude of activation showed a dose-dependent profile, with loss of regional specificity at the higher doses (100 and 300 nmol). ICV administration of Substance P was accompanied by a transient increase in mean arterial blood pressure (+15.20% for 100 nmol, $p < 0.05$), similar to that reported in previous studies [4]. Although changes in blood pressure can in principle affect cerebral haemodynamics, signal intensity and blood pressure changes did not show covariance. In 3 of the subjects no signal changes were observed; this may be due to oxidation of substance P, resulting in a loss of biological activity [5].

Conclusions

We have implemented procedures to study the phMRI response to neuroactive substances centrally administered via a permanent indwelling MR compatible cannula. We have applied this method to study for the first time the fMRI response to ICV infusion of substance P. This paradigm can also be used to probe the action of specific antagonists of Substance P receptors.

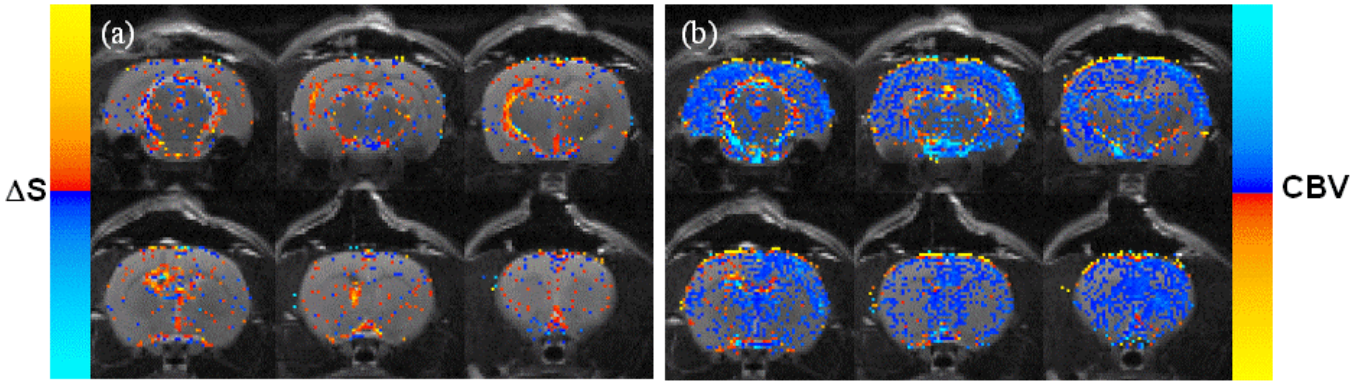

Figure 1: (a) Percentage signal changes during ICV infusion of 5 µl of saline (vehicle). (b) Significant CBV changes following ICV injection of 36 nmol Substance P (5-8min post-injection vs baseline) in a single animal. Both maps are thresholded at $p_u<0.05$.

References

[1] Menard J *et al*. (1999) Neuro Sci Behav Reviews **23** 591-613. [2] Mantyh PW *et al*. (2002) J Clin Psychiatry **63** 2-10. [3] Reese T *et al*. (2000) NMR Biomed **13** 43-49. [4] Unger *et al*. (1988) Circ Res **63** 812-20. [5] Merck Index (2001) 13, # **8947**.