Functional imaging of intracerebroventricular injection of Corticotropin-Releasing Factor (CRF) in the anaesthetised rat

A. Gozzi[1], A. J. Schwarz[1], T. Reese[1], V. Crestan[2], S. Bertani[2], G. Turrini[2], E. V. Merlo-Pich[3], J. J. Hagan[4], A. Bifone[1]

[1]Neuroimaging, GlaxoSmithKline Psychiatry Centre of Excellence for Drug Discovery, Verona, Italy, [2]Laboratory Animal Sciences, GlaxoSmithKline, Verona, Italy, [3]Discovery Medicine, GlaxoSmithKline Psychiatry Centre of Excellence for Drug Discovery, Verona, Italy, [4]Biology, GlaxoSmithKline Psychiatry Centre of Excellence for Drug Discovery, Verona, Italy

Introduction

Corticotropin-releasing factor (CRF), is the primary physiological regulator of basal and stress-induced peptide secretion from the anterior pituitary gland. In addition to its endocrine role, CRF and its receptors (CRF1 and CRF2) have been demonstrated to have a wide extra-hypothalamic distribution in the CNS [1]. Like other neuropeptides, CRF possesses distinct peripheral and central actions. Most of the endocrine, neurochemical and behavioural effects that occur after intracerebroventricular (ICV) injection of CRF are comparable to these accompanying stress responses [2]. However, the specific brain pathways through which CRF produces its varied behavioural effects remain unknown. In this study we used pharmacological MRI (phMRI) methods to map for the first time the haemodynamic response to acute ICV administration of CRF.

Methods

MR-compatible guide cannulae (Bilaney consultants, Germany) were stereotaxically implanted in to the right lateral ventricle of male Sprague-Dawley rats (n=11). After seven days of recovery, animals underwent surgery for MRI. This included tracheotomy for artificial ventilation, femoral artery and vein cannulation, for infusion of paralysing and contrast agent, and monitoring of blood gases. During image acquisition animal was kept anaesthetised with 0.8% halothane. 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. Following 5 image frames, the blood pool contrast agent Endorem (Guerbet, France) was administered i.v. (2.67 ml/kg). Following a 20-35 min delay, either CRF (3 μg [600 pmol]; n=8) or vehicle (saline; n=3) was manually infused ICV (5-9 μl in 60 s).

Results and discussion

ICV infusion of CRF produced a strong cardiovascular response, characterised by a steep and sustained increase in Mean Arterial Blood Pressure (MAP). Five animals showed a transient rCBV increase in the periventricular region of the diencephalon, corresponding to the choroid plexus, a highly vascularised area rich in CRF2 receptors [1]. No significant phMRI response was observed in other brain structures.

Central administration of CRF has been previously reported to induce marked cardiovascular alterations both in conscious and halothane-anaesthetised animals [4,5]. The detection of such a response was indicative of CRF biological activity under the experimental conditions of this study. Moreover, peripheral administration of CRF is known to produce *hypotension* due to a direct vasodilatory effect on blood vessel [4], thus suggesting that the CRF-dependent increase in MAP was centrally mediated. The activation of the periventricular area of the diencephalon is consistent with the relatively high concentration of CRF receptors, and with the exposure of receptors to the CSF.

Various hypotheses may explain the lack of detectable response in deeper structures. Halothane anaesthesia has been reported to produce a strong hypothalamic release of CRF in the rat [6]. Consequently, at the time of the ICV injection the CRF receptors may be already in a state of saturation, precluding further activation. Also, the large molecular size of CRF (M_w 4757) may limit its transport (diffusion) into brain tissue, while phMRI is sensitive to relatively rapid, transient signal changes in brain activity.

Conclusions

We have investigated the haemodynamic response to ICV challenge of CRF in the anaesthetised rat. A significant cardiovascular response, consistent with a central activity of CRF, was observed. FMRI response was localised in the periventricular region of the diencephalon, a region rich in CRF2 receptors.

References

[1] Chalmers T *et al.* (1995) J. Neurosc. **15** 6340-6320.
[2] Takahashi LK (2001) Neurosci and Behav Reviews **25** 627-636.
[3] Reese T *et al*. (2000) NMR Biomed **13** 43-49.
[4] Ritcher R *et al.* (1995) Peptides **16** 843-849.
[5] Kurosawa M *et al.* (1986) Brain Res **67** 250-257.
[6] Karuri AR *et al.* (1998) Brain Res Bul **47** 205-209.