Functional imaging of nicotine in the anaesthetised rat

A. Gozzi[1], A. J. Schwarz[1], T. Reese[1], S. Bertani[2], C. Heidbreder[3], A. Bifone[1]

[1]Department of Neuroimaging, Psychiatry CEDD, GlaxoSmithKline, Verona, Italy, [2]Laboratory Animal Sciences, Psychiatry CEDD, GlaxoSmithKline, Verona, Italy, [3]Department of Neuropsychopharmacology, Psychiatry CEDD, GlaxoSmithKline, Verona, Italy

Introduction

Nicotine, the major addictive component of tobacco, exerts several CNS-mediated effects on human and animal behaviours [1]. Nicotine's central activity is mediated by ligand-gated ion channel receptors that belong to the large nicotinic acetylcholine receptor family [2]. Pre-synaptic nicotinic receptors are known to modulate the release of several neurotransmitters involved in the reinforcement and reward pathway [3]. However, little is known about nicotine's effects on neuronal activity at a system level. Here we have applied phMRI methods to map for the first time the rCBV response to nicotine in the anesthetized rat. Moreover, we have investigated the effects of pre-treatment with the nicotinic receptor antagonist mecamylamine on nicotine-induced brain activity.

Methods

All experiments were carried out in accordance with Italian regulations governing animal welfare and protection and internal ethical review. Sprague-Dawley rats (250-350g) were scanned under halothane anaesthesia (induction 2.0%, maintenance level 0.8%). During the fMRI experiment, the 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). Arterial blood pressure was measured throughout the experiments and blood gas levels were sampled at several time-points. MRI data were acquired using a Bruker Biospec 4.7T system, a 72-mm birdcage resonator for RF transmit and a quadrature surface receive coil (Bruker, Ettlingen, Germany). The time series experiment comprised 90 time points using the RARE sequence: matrix 128x128; FOV 40mm; RARE factor 32; slice thickness 2mm; 8 contiguous coronal slices; 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. Three studies were performed: **(1)** acute i.v. nicotine challenge at two doses (1mg/kg [n=6] or 0.3mg/kg [n=7]) *vs*. vehicle (saline [n=6]). **(2)** i.p. mecamylamine challenge (1mg/kg [n=6], *vs*. vehicle (saline [n=3]). **(3)** pre-treatment with either mecamylamine (1mg/kg i.p., [n=7]) or vehicle (saline [n=9]) 30 minutes prior to i.v. nicotine challenge (1mg/kg, 30 min after i..p. injection). To rule out potential confounds arising from peripheral blood pressure changes elicited by nicotine, we have performed additional experiments with norepinephrine, a potent non-brain-penetrant vasopressor, at a dose that mimics the nicotine cardiovascular response (0.5 µg/kg,, i.v, [n=4]). Image analysis comprised co-registration of the image data from all animals to the same subject, masking out of non-brain tissue, conversion of time series' to rCBV. The nicotine response was quantified by pixel-wise multiple linear regression. Group comparisons were performed by t-tests between appropriate groups of contrast images. The statistical threshold of effect was determined using the Benjamini-Hochberg procedure with a false discovery rate (FDR) of q=0.05. RCBV time courses were extracted *a posteriori* for specific regions of interest (ROIs) based on correspondence between the anatomical images and an anatomical atlas.

Results

Acute infusion of nicotine (1 mg/kg) induced significant rCBV increases in the amygdala, the caudal-ventral hippocampus, endopyriform nucleus, and several cortical structures (cingulate, medial prefrontal, insular, rhinal cortices, Figure 1). The lower dose of nicotine (0.3 mg/kg) did not produce a statistically significant rCBV response. Analysis of the time-course profile of activation (Figure 2) showed a fast increase in rCBV peaking approximately 2 min after injection, followed by a gradual return to pre-injection baseline values (ca. 10 minutes post injection). Prior treatment of rats with the non-competitive nicotinic acetylcholine receptor antagonist mecamylamine (1 mg/kg, i.p.) strongly attenuated nicotine-induced rCBV response (Figure 2). Mecamylamine alone (1 mg/kg i.p.) did not show detectable effects on rCBV. Nicotine injection induced only a slight and transient increase in arterial blood pressure, which was significant only 1 minute post injection (+27 %, $p<0.01$). Pre-treatment with mecamylamine did not affect amplitude and duration of peripheral blood pressure changes. Acute administration of norepinephrine (0.5 µg/kg, i.v.) gave rise to a blood pressure increase similar to that observed with by 1 mg/kg nicotine ($p>0.01$, all time-points). Animals challenged with norepinephrine did not exhibit detectable rCBV changes, thus ruling out potential confounding peripheral effects.

Discussion

The brain structures showing the strongest rCBV response correspond with regions of high density of the α7 subtype nicotinic acetylcholine receptor [2,4]. Moreover, the activation pattern shows a remarkable degree of similarity with the distribution of c-Fos-like immunoreactive nuclei following acute nicotine challenge [5]. Pre-administration of mecamylamine has been reported to block the behavioural effects of nicotine [1]. Consistently, mecamylamine prevented rCBV changes induced by nicotine. The results of this study help elucidate the functional substrate of nicotine, and pave the way to the use of nicotine as a pharmacological tool in functional MRI studies of drug-dependence.

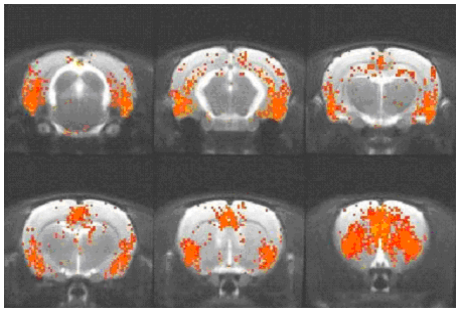

***Figure 1**: Group response map to intravenous challenge with nicotine 1 mg/kg (anterior 6 slices). Orange areas indicate* increased *rCBV response compared to vehicle.*

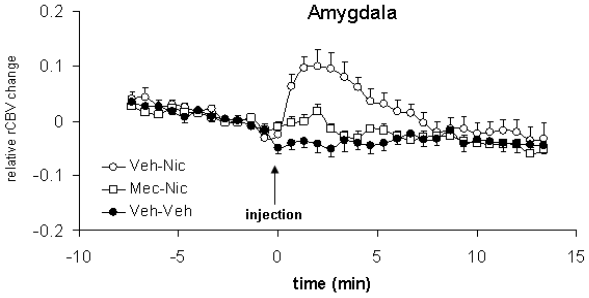

***Figure 2**: RCBV changes in the amygdala following nicotine challenge in vehicle (Veh-Nic) or mecamylamine (Mec-Nic) pre-treated rats. Data from control animals (Veh-Veh) are included for comparison. Time courses are shown as mean±SEM across animals. The negative trend in rCBV curves is due to contrast agent washout.*

References

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