PhMRI effects of the M4 muscarinic receptor acetylcholine positive allosteric modulator VU0152100 on dopaminergic activity

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Introduction

Pharmacologic MRI (phMRI) has previously been used to assess the effects of neuroactive drugs *in vivo* at a systems level. Recently, we reported that the selective M4 muscarinic acetylcholine receptor positive allosteric modulator VU0152100 suppresses amphetamine-induced hyperlocomotion [1], a preclinical model considered to be predictive of the antipsychotic efficacy of a compound. In this study, we used cerebral blood volume (CBV) phMRI to quantify the regional effects of selective M4 potentiation with VU0152100 on amphetamine-induced increases in brain activity and modifications of functional connectivity between key dopaminergic targets and downstream areas in rat brain. We also report separate microdialysis data that corroborate the striatal phMRI results.

Methods

MR studies were performed on a 9.4T scanner (Varian Inc., Palo Alto, CA) using a Doty Litz 38 coil. Structural and functional images were acquired with a T2-weighted fast spin echo (fse) sequence. High resolution fse anatomical images were collected (TR = 2550 ms, TE_{eff} = 40 ms, acquisitions = 2, matrix = 256 × 256, 11 slices, 1.5mm thick) to facilitate registration. After acquiring 10 initial pre-contrast images (TR = 2500 ms, TE_{eff} = 36 ms, acquisitions = 2, matrix = 64 × 64, one image every 41.6 s), MION (20 mg/kg, i.v.; BioPal, Worcester, MA) were injected. VU0152100 (56.6 mg/kg i.p.) or vehicle (Veh) was then administered. The post-MION functional scan consisted of a 15 min baseline, amphetamine (Amph, 1 mg/kg, i.p.) or Veh administration 30 min after VU0152100 injection; then images were acquired for another 45 min. Adult male Sprague-Dawley rats were intubated and mechanically ventilated (0.88% isoflurane, O_2:N_2O 1:2) under neuromuscular blockade (pancuronium bromide 1 mg/kg, i.p.). Heart rate, respiration, temperature, and end-tidal CO_2 were monitored. The following were scanned (n = 10-11/group): Veh/Veh, Veh/Amph, and VU0152100 (56.6 mg/kg)/Amph (1 mg/kg). In a separate study, VU0152100 (56.6 mg/kg) or Veh was the challenge. Fractional CBV change was calculated as $[\ln (S(t)/S_o)]/[\ln (S_o/S_{pre})]$, where S_o is the average baseline signal and S_{pre} is the mean pre-MION signal [2]. ΔCBV/CBVo time courses were extracted from regions of interest (ROIs). Inter-regional correlations in the Amph response were investigated by computing the Pearson linear correlation coefficient (CC) between ΔCBV responses for each ROI pair [3] in the treated and untreated groups. Each CC, r, was converted to a z-score ($z = \ln[(1+r) / (1-r)] / [2*(1 / (N-3))0.5]$) and thresholded at $p<0.05$. Permutation analysis was used to identify correlations that were different (Δz, $p < 0.05$) between the two groups. Microdialysis studies evaluated the effects of VU0152100 on changes in extracellular dopamine (DA) levels in the striatum of awake rats (n = 4-5/group). VU0152100-induced changes in blood pressure were measured separately (n = 5-6/group).

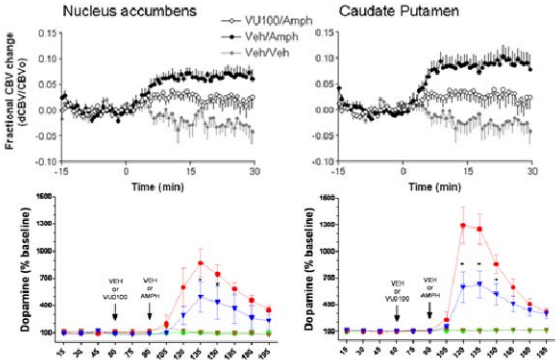

Fig. 1. VU0152100 suppresse Amph-induced CBV changes in the nucleus accumbens and caudate putamen (top row). Pretreatment with VU0152100 decreases Amph-induced extracellular dopamine levels (bottom row).

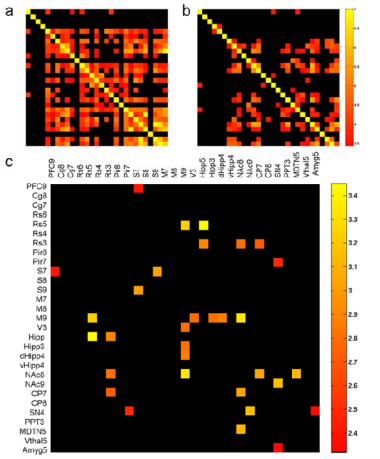

Fig. 2. Significant correlations from Amph (a) are altered by VU1052100 pretreatment (b). Differences ($p < 0.05$) between (a) and (b) are shown in (c).

Results

VU0152100 pretreatment significantly blunted Amph-evoked CBV increases in the nucleus accumbens (NAc) and caudate-putamen (CP) (Fig. 1) as well as in the thalamus and hippocampus, compared to the untreated (Veh/Amph) group. There was no significant response to Veh alone. In separate microdialysis studies, VU0152100 pretreatment reduced Amph-induced increases in extracellular DA in NAc and CP (Fig. 1). VU0152100 alone transiently decreased blood pressure with no effect on heart rate, had no detectable phMRI effect, and no effect on striatal DA levels. Functional connectivity analyses revealed multiple inter-regional correlations in the Veh/Amph group (Fig. 2a), with fewer correlations found in VU0152100-pretreated rats (Fig. 2b). Significant differences between the two groups were identified through permutation analysis, including correlations of retrosplenium-hippocampus, NAc-motor cortex, retrosplenium-motor cortex, NAc-substantia nigra, and NAc-mediodorsal thalamus (Fig. 2c).

Discussion

These preclinical neuroimaging results, along with the microdialysis data, support potentiation of endogenous cholinergic activity at M4 as a novel antipsychotic mechanism through which dopaminergic activity can be modulated without directly targeting DA receptors. PhMRI based on CBV measurements provides a unique method for assessing regional effects and interactions.

References

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