Characterizing the Differential Effects of Selective Cannabinoid Agonists on Brain Activity in Awake Rats Using Pharmacological MRI

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Introduction
Cannabis has been used to relieve some of the symptoms associated with CNS disorders such as multiple sclerosis and pain [1]. The pharmacological effects of cannabis and its derivatives, collectively known as cannabinoids (CB), are mainly mediated via two G-protein coupled receptor subtypes: CB1, which are found predominantly in CNS, and CB2, which are presently thought to be primarily located in immune cells and absent from CNS [1]. CB receptors are currently under investigation as potential therapeutic targets for a variety of conditions. Recent publications indicate that selective activation of CB2 receptors produces analgesia without the undesirable psychotrophic side effects associated with activation of CB1 receptors [2-4]. Thus, it is important to demonstrate functional selectivity in vivo prior to developing CB2-selective agonists for pain. Therefore, we performed pharmacological MRI (phMRI) [5] in awake rats to examine the effects of non-selective CB1/CB2 (A-834735 [1-(Tetrahydro-pyran-4-ylmethyl)-1H-indol-3-yl]-[2,2,3,3-tetramethyl-cyclopropyl]-methanone), [6]) and CB2-selective (AM1241) agonists on functional brain activity. In addition, pharmacological specificity was determined using either CB1-selective (rimonabant) or CB2-selective (AM-630) antagonists.

Materials and Methods
Male SD rats (~300 g) were imaged under awake conditions. Prior to imaging, rats were acclimated to a dedicated animal holder that is integrated with a dual RF coil system (INSL, Worcester MA) for imaging. The training procedure required four different sessions of various durations on separate days [7]. Experiments were carried out on a 7T Bruker scanner using a RARE sequence with imaging parameters: TR/TE = 3200/70 ms, RARE factor = 16, pixel size = 300 × 300 µm², slice thickness = 1.5 mm, 13 contiguous coronal slices, and average = 2. The phMRI protocol included (i) 4 min baseline acquisition, (ii) a bolus injection of USPIO contrast agent (SH U 555 C, 15 mg Fe/kg, iv, Schering AG, Germany), (iii) 15 min pre-drug baseline, (iv) a 5 min vehicle or drug infusion (PEG 400 vehicle, A-834735 at 0.3, 1, 3 µmol/kg iv, rimonabant at 13 µmol/kg iv, AM630 at 6 µmol/kg iv, or AM1241 at 30, 100 µmol/kg iv), and (v) 25-min post-drug acquisition. For antagonist blockade experiments, rats were pre-treated with either rimonabant at 13 µmol/kg ip or AM630 at 6 µmol/kg ip approximately 40 min before the infusion of A-834735 at 1 µmol/kg iv. Data analyses were performed using AFNI [8]. Cross-correlation coefficients (cc) between time-course raw data and a step function were calculated. The statistical parameter (z-score) was derived from cc and later used to create group average activation maps.

Results and Discussion
Figure 1 shows the group average activation maps obtained from rats intravenously infused with PEG 400 (Fig. 1A), A-834735 at 0.3, 1, or 0.3 µmol/kg (Fig. 1B-D, respectively), rimonabant at 13 µmol/kg (Fig. 1E), and AM630 at 6 µmol/kg (Fig. 1F). No significant activation was observed from vehicle-, rimonabant-, or AM630-treated groups, whereas A-834735 produced a dose-related region-specific activation of brain regions that agrees well with published autoradiographic CB1 receptor binding maps [9]. Pretreatment with rimonabant (Fig. 1G), but not with AM630 (Fig. 1H), abolished the effects of A-834735 at 1 µmol/kg, indicating an effect mediated by CB1 receptor alone. It was previously reported that significant increases in BOLD signal were observed in brain regions associated with motor function (striatum), reward (ventral tegmental area and nucleus accumbens), and pain (periaqueductal grey) in rats treated with a CB1 agonist (HU210) [10], and human PET studies revealed that tetrahydrocannabinol (THC) elicited significant increases in rCBF in frontal, insular, and anterior cingulate regions [11]. Our phMRI results are consistent with these previous findings. Interestingly, AM1241 at 10 and 30 µmol/kg did not elicit any significant changes in brain activity (Fig. 1I-J, respectively). As the presence of CB2 receptors in the brain remains controversial [1, 4], our data suggest that, if CB2 receptors are expressed, they are not functional under normal physiological conditions. In summary, we have shown that differential effects of CB1 and CB2 agonists on brain activity using phMRI in awake rats can provide value to help characterize psychotropic side effects associated with CB1 activation while developing CB2-mediated analgesics.

References