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Oral haloperidol or olanzapine intake produces distinct and region-specific increase in cannabinoid receptor levels that is prevented by high fat diet

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ABSTRACT

Clinical studies show higher levels of cannabinoid CB1 receptors (CB1R) in the brain of schizophrenic patients while preclinical studies report a significant functional interaction between dopamine D2 receptors and CB1Rs as well as an upregulation of CB1Rs after antipsychotic treatment. These findings prompted us to study the effects of chronic oral intake of a first and a second generation antipsychotic, haloperidol and olanzapine, on the levels and distribution of CB1Rs in the rat brain. Rats consumed either regular chow or high-fat food and drank water, haloperidol drinking solution (1.5 mg/kg), or olanzapine drinking solution (10 mg/kg) for four weeks. Motor and cognitive functions were tested at the end of treatment week 3 and upon drug discontinuation. Two days after drug discontinuation, rats were euthanized and brains were processed for in vitro receptor autoradiography. In chow-fed animals, haloperidol and olanzapine increased CB1R levels in the basal ganglia and the hippocampus, in a similar, but not identical pattern. In addition, olanzapine had unique effects in CB1R upregulation in higher order cognitive areas, in the secondary somatosensory cortex, in the visual and auditory cortices and the geniculate nuclei, as well as in the hypothalamus. High fat food consumption prevented antipsychotic-induced increase in CB1R levels in all regions examined, with one exception, the globus pallidus, in which they were higher in haloperidol-treated rats. The results point towards the hypothesis that increased CB1R levels could be a confounding effect of antipsychotic medication in schizophrenia that is circumveneted by high fat feeding.

1. Introduction

Increased cortical and subcortical cannabinoid CB1 receptor (CB1R) binding has been reported in human schizophrenia studies. In vivo brain imaging shows higher CB1R levels in frontal, temporal, parietal, medial cortices, the n. accumbens (NAC) (Ceccarini et al., 2013), and the pons (Wong et al., 2010) of schizophrenic patients, in agreement with post-mortem receptor binding studies also showing CB1R upregulation in dorsolateral prefrontal and cingulate cortices in schizophrenia patients (Dean et al., 2001; Zavitsanou et al., 2004; Newell et al., 2006; Dalton et al., 2011; Jenko et al., 2012).

Studies have also shown that CB1R levels are modulated by the dopaminergic system, in particular dopamine D2 receptors (D2R). Genetic ablation of D2Rs leads to a marked upregulation of cannabinoid CB1Rs in the cerebral cortex, the striatum, and the NAC (Thanos et al., 2011). Treatment with haloperidol, a first generation antipsychotic with high affinity for D2Rs, also leads to higher CB1R levels and CB1R activity in the basal ganglia (Andersson et al., 2005). On the other hand, studies have shown that haloperidol decreases frontal cortical CB1R protein levels in women (Uriguen et al., 2009) and CB1R-mediated signaling in the frontal cortex of female rats (Wiley et al., 2008). CB1R levels increase in the hippocampus and the amygdala after risperidone treatment (Secher et al., 2010) and in the NAC after prolonged olanzapine withdrawal (Sundram et al., 2005). On the other hand, a selective decrease in CB1Rs in the arcuate nucleus and the dorsal vagal complex was shown after olanzapine treatment in female rats (Weston-Green et al., 2012). Still, other studies show no effects of haloperidol (Sundram et al., 2005) or olanzapine (Llorente-Berzal et al., 2012; Lazzari et al., 2017) treatment on CB1R levels. If antipsychotic treatment modulates brain CB1R levels, it would be necessary to study brain regions and circuits in which CB1Rs are most affected by antipsychotics, in order to clarify the potentially confounding effect of antipsychotic medication in humans.

Second generation antipsychotic treatment is associated with overeating, weight gain, and related metabolic disorders (Eder et al., 2001; Gothelf et al., 2002; Minet-Ringuet et al., 2006; Allison et al., 2009;

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Muller et al., 2010; van der Zwaal et al., 2010; van der Zwaal et al., 2012; Daurignac et al., 2015). In addition, food intake and body weight regulation are directly related to the endocannabinoid system, since genetic ablation and pharmacological antagonism of CB1Rs lead to leanness and suppress food intake (Verty et al., 2004; Salamone et al., 2007; de Kloet and Woods, 2009; Pang et al., 2011; Lazzari et al., 2012; Manca et al., 2013; Mastinu et al., 2013). Most interestingly, CB1R antagonism normalizes olanzapine-induced changes in enzymes and metabolic parameters expressed in brain, liver, and blood (Lazzari et al., 2017). If antipsychotic treatment increases brain CB1R levels, it would be important to identify whether this increase is related to the amount and type of food intake.

Based on the above, we studied the effects of haloperidol or olanzapine oral intake (1 month) on CB1R levels in the male rat brain. Effects of food intake (standard rodent chow vs. high fat (HF)) were also considered. A battery of motor and non-motor tasks was applied to the rats during- and post-treatment to assess the behavioral state of the animals.

2. Methods

2.1. Animals

Sixty adult male Sprague Dawley rats (Charles Rivers, Wilmington, MA, USA) were handled daily for two weeks prior to collection of baseline measurements. Animals were individually housed and kept on a reverse 12:12 L/D cycle with lights off at 07:00. Food and water were provided ad libitum. Animal weight, food, and fluid intake were measured daily. Animals were randomly assigned to 1 of 2 food groups (regular laboratory chow or regular chow + HF food) and 1 of 3 treatments (water, haloperidol solution (1.5 mg/kg), or olanzapine solution (10 mg/kg)). All experiments were conducted and approved by the National Academy of Sciences Guide for the Care and Use of Laboratory Animals (NAS, 1996) and the University at Buffalo IACUC.

2.2. Experiment outline

Haloperidol (H1512, Sigma Aldrich, US) and olanzapine (O253750, Toronto Research Chemicals, Canada) drinking solutions were prepared by dissolving the powder in 0.1 N HCl, diluting with water, and fixing the pH to 7 with very dilute NaOH (Terry et al., 2007). The concentration of the solution was based on the animals' average fluid intake of the previous day and on their body weight in order to formulate a drug dose of 1.5 mg/kg/day for haloperidol and 10 mg/kg/day for olanzapine. Drug solutions were prepared on a daily basis and no precipitate was observed in the bottles. Olanzapine has been previously shown to be stable in drinking water for at least 96 h (Terry et al., 2008). Drug doses were based on previous studies showing that at these doses the drugs, administered orally and chronically, have no more than 80% dopamine D2 receptor occupancy, thus being therapeutically relevant and without motor side-effects (Kapur et al., 1999; Kapur et al., 2003; Terry et al., 2005; Barth et al., 2006).

2.2.1. Drugs

The experimental timeline is depicted in Fig. 1, below. Drug treatment lasted 28 days, from Day 0 to Day 27. High fat (HF) food (Test Diets 58Y1 with 35% fat vs. regular chow Test Diets 5001 with 13% fat) was added to the Chow + HF groups on Day 2. In this group, both foods were offered in the food container, separated with a divider. Food position was switched every 3–4 days to prevent position bias; however, all rats had 100% preference for the HF food from the first day it was presented until the end of the experiment. Motor and cognitive functions were assessed during treatment, in the AM (rats' dark cycle, active phase, under red light) of Days 20 and 21, and post-treatment, during the AM of Days 28 and 29. The rats were euthanized in the AM of Day 30.

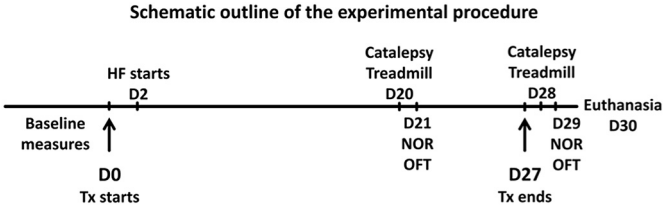

Fig. 1. Schematic outline of the experimental procedure. D: day; Tx: treatment; NOR: novel object recognition test; OFT: open field test; HF: high-fat food offered along with standard diet and preferred 100%.

2.3. Behavioral assessments

2.3.1. Catalepsy test

The rats were placed on a grid that was inclined at 60°. We measured the rats' latency to perform the first ambulatory movement with all four legs. Time is expressed in s.

2.3.2. Treadmill

To assess motor coordination, strength, and endurance, we measured the maximum speed of the treadmill belt that the rats were able to follow. One day before the test, the rats were placed on the treadmill at the lowest speed to habituate to the setup. On test day, the belt speed ranged from 22.5 cm/s to 50 cm/s and increased by 2.5 cm/s every 1 min. When a rat stopped running for more than 10s, it was removed from the treadmill belt and the speed of the belt at that time was recorded.

2.3.3. Open field test

The open field test was performed in a cubic arena (edge: 40 cm) that records horizontal and vertical motion through a series of cross beams that are positioned 5 cm apart, allowing for 2.5 cm resolution (Tru Scan System, Coulbourn Instruments, PA, USA). The animal was placed in the center of the open field arena and each session lasted for 60 min. We report ambulatory distance (cm) and vertical activity (rearing, number of vertical counts).

2.3.4. Novel object recognition

The novel object recognition test, an example of a non-matching to target test (Ennaceur and Delacour, 1988), was employed to assess short- and long-term memory at intertrial intervals T2 = 5 min and T3 = 1 h, respectively. During trial T1, the rats were presented 2 identical objects [A, A] in a 40 cm edge cubic arena and left to explore them for 5 min. Five min after the end of T1, they were re-placed in the arena (trial T2) and left to explore, for 5 min, objects [A, B] that had been placed in the same positions as [A, A]. One hour later, the rats were placed once more in the arena (trial T3) where 2 objects [A, C] had been placed in the same positions as in T1 and T2. The rats were videotaped with a camera that was hanging from the ceiling. Object exploration was defined as approaching the object at 2 cm with the head facing the object, sniffing, whisking, or touching the object with the forepaws. Exploration of the novel (N) and familiar (F) objects were measured in s. Object Discrimination Index (DI) is expressed as the ratio (N − F)/(N + F). Total object exploration time is also reported in s. Two different sets of objects, [A, B, C] and [A′, B′, C′], were used when the rats were tested during- and post-treatment, respectively.

2.4. Receptor studies

2.4.1. Tissue preparation

Rats were decapitated after isoflurane anesthesia, brains were rapidly extracted on ice, flash-frozen in methylbutane (− 40 °C), and stored at − 70 °C. Fifteen μm-thick sections, prepared with a cryostat (Leica CM30505), were thaw-mounted on clean charged glass slides. Every 3rd section was collected and the tissue was divided into 5 sets,