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Acute administration of Δ^9 tetrahydrocannabinol does not prevent enhancement of sensory gating by clozapine in DBA/2 mice

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ABSTRACT

Despite high rates of marijuana abuse in schizophrenia, the physiological interactions between tetrahydrocannabinol (THC) and antipsychotic medications are poorly understood. A well-characterized feature of schizophrenia is poor gating of the P50 auditory-evoked potential. This feature has been translationally modeled by the DBA/2 mouse, which exhibits poor suppression of the P20–N40 AEP, the rodent analog of the human P50. Previous work has demonstrated that this deficit is reversed by the antipsychotic clozapine. It is unknown, however, if this effect is altered by THC administration. Using a conditioning–testing paradigm with paired auditory stimuli, the effects of clozapine and dronabinol (a pharmaceutical THC formulation) on inhibitory P20–N40 AEP processing were assessed from *in vivo* hippocampal CA3 recordings in anesthetized DBA/2 mice. The effects of clozapine (0.33 mg/kg) and dronabinol (10 mg/kg) were assessed alone and in combination (0.33, 1 or 1.83 mg/kg clozapine with 10 mg/kg dronabinol). Improved P20–N40 AEP gating was observed after acute administration of 0.33 mg/kg clozapine. Co-injection of 0.33 mg/kg clozapine and 10 mg/kg THC, however, did not improve gating relative to baseline. This effect was overcome by higher doses of clozapine (1 and 1.83 mg/kg), as these doses improved gating relative to baseline in the presence of 10 mg/kg THC. 10 mg/kg THC alone did not affect gating. In conclusion, THC does not prevent improvement of P20–N40 gating by clozapine.

1. Introduction

A daunting challenge facing clinicians who attempt to treat patients with schizophrenia is the large number of comorbidities that present alongside the illness, including increased rates of diabetes, heart disease, obesity, and substance abuse (Buckley et al., 2009; Mitchell et al., 2013; Volkow, 2009). Substance abuse in particular is one of the most prevalent comorbid conditions, with nearly half of patients presenting with a lifetime history of substance abuse disorders (Volkow, 2009). One of the most commonly abused drugs in schizophrenia is cannabis, with 50% or more of patients dependent on the substance according to DSM-IV criteria, and up to 80% of patients reporting current or recent use, depending on the study (Volkow, 2009). The relationship between cannabis use and schizophrenia is complex and not well-understood. A meta-analysis by Moore et al. (2007) demonstrated higher risk for developing schizophrenia in subjects who used marijuana in the past. Other studies have shown that marijuana use may exacerbate existing symptoms (e.g. Bersani et al., 2002). In contrast, other groups have argued that cannabis use is not causally related to the illness (Costain, 2008; Hambrecht and Hafner, 2000).

An added concern regarding cannabis abuse in schizophrenia is how tetrahydrocannabinol (THC), the principal psychoactive constituent of marijuana, may neuropharmacologically interact with antipsychotics and alter their therapeutic effectiveness. Cannabis can worsen positive symptoms in medicated patients (Foti et al., 2010; Zammit et al., 2008), suggesting it may dampen antipsychotic effectiveness. However, THC may improve negative symptoms, reduce stress and anxiety, improve social outcomes, and even improve cognition in patients (Potvin et al., 2006; Rabin et al., 2011; Salyers and Mueser, 2001; Yucel et al., 2012). The ineffectiveness of antipsychotic medication at treating negative and cognitive symptoms has led some researchers to speculate that patients use marijuana as a form of "self-medication" (Khantzian, 1997; Schneier and Siris, 1987).

Although the symptomatic correlates of cannabis administration are clinically and therapeutically informative, the underlying heterogeneity of schizophrenia makes it problematic to neuropharmacologically interpret the effects of THC in the illness by measuring symptoms alone. To better understand how THC affects brain function, researchers have used techniques such as scalp electroencephalography (EEG) to measure neurophysiological responses to stimuli. Starting with the observations of Bleuler (1911) as well as McGhie and Chapman (1961), researchers have frequently observed that schizophrenia patients are hyper-responsive to sensory stimulation, most commonly in the auditory domain. Patients are particularly impaired in the ability

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to "tune out" repetitive auditory stimuli such as a fan blowing or a clock ticking. Physiologically, this phenotype may be associated with reduced "P50" gating in the illness. P50-gating is an electrophysiological phenomenon in which individuals reduce their early (~50 ms post-stimulus) neuronal response to the second of a pair of repeated identical auditory clicks. This suppression is typically quantified by the "P50 ratio", or ratio of the magnitude of the second response (S2) over the first response (S1), i.e. S2/S1. Healthy subjects, on average, present with P50 ratios less than 0.50, whereas patients with schizophrenia often present with ratios of 0.75 or greater (Adler et al., 1982). This abnormality may be correlated with impaired selective attention (Smucny et al., 2013).

One advantage to studying P50 gating is its translational applicability. By recording directly from the hippocampal CA3 subfields of various mouse strains, gene knockouts, and pharmacologic models, researchers have been able to better characterize the molecular underpinnings that are involved in auditory P50 gating in a P50 analog called the P20–N40 auditory evoked potential. The DBA/2 mouse, a strain that displays multiple putative symptom analogs of schizophrenia (e.g. cognitive dysfunction) and shows reduced α7 nicotinic receptor expression, shows particularly poor hippocampal P20–N40 gating relative to most other strains (Stevens and Wear, 1997). Interestingly, this deficit can be reversed by the atypical antipsychotic clozapine (Simosky et al., 2003). This clozapine effect is blocked by nicotinic receptor (NAChR) antagonists, suggesting that clozapine may improve gating through a cholinergic mechanism (Simosky et al., 2003). Specifically, it is hypothesized that activation of α7 NAChRs facilitates the release of the inhibitory neurotransmitter gamma-aminobutyric acid, enhancing suppression of neuronal response (Miwa et al., 2011; Simosky et al., 2003).

Despite the widespread use of cannabis in schizophrenia, its effects on auditory gating, particularly when co-administered with antipsychotics, are not well understood. The goal of the present study was to better understand the interaction between acute THC and clozapine administration in mice that show poor gating (analogous to schizophrenia patients) at baseline. To that end, this study examined the effects of dronabinol (Marinol), a pharmaceutical formulation of Δ^9 tetrahydrocannabinol (THC), on clozapine-induced improvement of P20–N40 gating in DBA/2 mice. We hypothesized that THC would impair the ability of clozapine to improve gating, based on its demonstrated ability to reduce GABA release (Katona et al., 1999) and attenuate pharmacologic modulation of inhibitory neurotransmission.

2. Materials and methods

2.1. Mice

Male DBA/2 mice were purchased from Harlan (Indianapolis, IN) and group housed in shoe-box cages on Aspen chip bedding until recording. Mice were 7–10 weeks old at the time of recording. Food (Purina Rodent Chow) and water was available ad libitum during housing. Animals were maintained under a 12-hour light/dark cycle (lights off at 6 pm). "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, revised 1985) were followed. The Institutional Animal Care and Use Committee of the Denver Veterans Affairs Medical Center and/or the University of Colorado Anschutz Medical Campus approved the experimental protocols.

2.2. In vivo hippocampal P20–N40 recordings

Adult DBA/2 mice (20–25 g; $n = 46$) were anesthetized with 400 mg/kg chloral hydrate i.p. and 400 mg/kg pyrazole i.p. Mice were then placed in a stereotaxic apparatus, and hollow ear bars with attached earphones were positioned adjacent to the mouse's ears. Body temperature was maintained at 37 °C with a heating pad. A teflon-coated stainless-steel recording electrode (0.127 mm in diameter) was inserted into the pyramidal layer of hippocampal area CA3 at 1.8 mm posterior to bregma, 2.7 mm lateral to the midline and 1.5–1.7 mm below the surface of the dura (Paxinos and Franklin 2001). Final location was identified by the presence of complex action potentials typical of hippocampal pyramidal neurons (Miller and Freedman, 1995). A similar reference electrode was placed on the dura, contralateral to the recording electrode, just anterior to bregma. Tones (3000 Hz, 10 ms, 70 dB SPL) were presented in pairs separated by 500 ms with 10 s between tone pairs. A baseline period of 50 ms preceded each tone pairing. Recordings were segmented from −50 to 350 ms after stimulus onset. Eighteen sets (5 m duration/set) of averaged responses to 16 tone-pairs were recorded per animal. Of these eighteen sets, 6 sets were taken before drug administration as a baseline, and the remaining 12 were taken after drug administration. Thus, responses were measured for up to one hour post injection.

Each averaged response was amplified 1000 times, bandpass filtered between 1 and 500 Hz, and sent to a computer software program (SciWorks, DataWave, Loveland, CO) for data analysis and storage. The maximum negativity between 20 and 60 ms after each auditory stimulus was selected (i.e. the P20–N40) and measured relative to the preceding positivity. This complex has been shown to have less variability than either component alone (P20 or N40) (Hashimoto et al., 2005).

The number of mice per group were as follows: for 0.33 mg/kg clozapine (only) $n = 9$, for 0.33 mg/kg clozapine + 10 mg/kg dronabinol $n = 9$, for 1 mg/kg clozapine + 10 mg/kg dronabinol $n = 9$, for 1.83 mg/kg clozapine + 10 mg/kg dronabinol $n = 9$,or 3.33 mg/kg clozapine + 10 mg/kg dronabinol $n = 2$, and for 10 mg/kg dronabinol (only) $n = 8$.

2.3. Drugs

Clozapine (0.33, 1, 1.83, or 3.33 mg/kg) was dissolved in saline (pH ~5.5) (80 μl for every 20 g of body weight) and injected i.p. Dronabinol (10 mg/kg) was dissolved in sesame oil (80 μl for every 20 g of body weight) and injected i.p. One drug was injected immediately after the other.

2.4. Statistical analysis

For these studies, the most relevant components of the P20–N40 AEP response of the DBA/2 mice were the amplitude of the response to the first auditory stimulus (the S1 amplitude) and the amplitude of the response to the second auditory stimulus (the S2 amplitude). The magnitude of inhibitory processing of the S2 response was determined by calculating the S2/S1 ratio, or the S2 amplitude divided by the S1 amplitude. Effective gating is characterized by S2/S1 ratios significantly lower than 1. The S2/S1 ratio is the predominant measure used to assess the efficacy of a drug in normalizing deficits in P20–N40 AEP inhibitory processing.

For these experiments, 6 sets of baseline recordings (16 trials/set, with each set 5 min apart) were followed by up to 12 identical sets of post-drug administration recordings. The effects of drug(s) administration on the P20–N40 AEP (S1 amplitude, S2 amplitude, S2/S1) were analyzed using repeated measures analyses of variance (ANOVA), with time as a within-subjects factor and dose/drug combination as a between-subjects factor. *A priori* hypotheses comparing the effects of doses/drugs were tested using the main effect of dose/drug as well as the dose/drug * time interaction. In addition, *a priori* contrast analyses using Fisher's LSD post-hoc tests were conducted for each dose to compare the mean of the "baseline" (pre-drug) AEP measures and the mean of the "treatment" (post-drug) AEP measures. Using the *a priori* contrast analysis maximized the statistical power for detecting expected baseline versus treatment differences (statistical power was preserved because this test was run prior to making multiple comparisons with the other statistical tests used). For each ANOVA, post-hoc