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Acetaminophen differentially enhances social behavior and cortical cannabinoid levels in inbred mice

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ABSTRACT

Supratherapeutic doses of the analgesic acetaminophen (paracetomol) are reported to promote social behavior in Swiss mice. However, we hypothesized that it might not promote sociability in other strains due to cannabinoid CB_1 receptor-mediated inhibition of serotonin (5-HT) transmission in the frontal cortex. We examined the effects of acetaminophen on social and repetitive behaviors in comparison to a cannabinoid agonist, WIN 55,212-2, in two strains of socially-deficient mice, BTBR and 129S1/SvImJ (129S). Acetaminophen (100 mg/kg) enhanced social interactions in BTBR, and social novelty preference and marble burying in 129S at serum levels of $\geq$70 ng/ml. Following acetaminophen injection or sociability testing, anandamide (AEA) increased in BTBR frontal cortex, while behavior testing increased 2-arachidonyl glycerol (2-AG) levels in 129S frontal cortex. In contrast, WIN 55,212-2 (0.1 mg/kg) did not enhance sociability. Further, we expected CB_1-deficient (+/−) mice to be less social than wild-type, but instead found similar sociability. Given strain differences in endocannabinoid response to acetaminophen, we compared cortical CB_1 and 5-HT_{1A} receptor density and function relative to sociable C57BL/6 mice. CB_1 receptor saturation binding (Bmax = 958 ± 117 fmol/mg protein), and affinity for [^{3}H] CP55,940 (K_D = 3 ± 0.8 nM) was similar in frontal cortex among strains. CP55,940-stimulated [^{35}S] GTPγS binding in cingulate cortex was 136 ± 12, 156 ± 22, and 75 ± 9% above basal in BTBR, 129S and C57BL/6 mice. The acetaminophen metabolite para-aminophenol (1 μM) failed to stimulate [^{35}S] GTPγS binding. Hence, it appears that other indirect actions of acetaminophen, including 5-HT receptor agonism, may underlie its sociability promoting properties outweighing any CB_1 mediated suppression by locally-elevated endocannabinoids in these mice.

Abbreviations: 2-AG, 2-arachidonyl glycerol; 8-OH-DPAT, 8-Hydroxy-2-(di-n-propylamino)tetralin; ACM, acetaminophen; AEA, anandamide; AM404, N-arachidonyl-phenolamine; ANOVA, analysis of variance; CB, cannabinoid; DMSO, dimethyl-sulfoxide; FAAH, fatty acid amide hydrolase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; HPLC, high performance liquid chromatography; OEA, oleoylethanolamide; PCR, polymerase chain reaction; TRPV1, transient receptor potential vanilloid 1 cation channels; Tris, Tris(hydroxymethyl) aminomethane; WIN, WIN 55,212-2.

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1. Introduction

Deficits in social interaction such as social anxiety, withdrawal, and inattentiveness are symptoms of many psychiatric conditions, including autism, schizophrenia, depression and post-traumatic stress disorder (Derntl et al., 2011; Feldman and Vengrober, 2011; Pinkham et al., 2008). Behaviors paralleling sociability impairments are inherent in several inbred strains of mice, including BTBR and 129S1/SvImJ (Defensor et al., 2011; Moy et al., 2007; Spencer et al., 2011). BTBR mouse social behavior is sensitive to changes in serotonin (5-HT) neurotransmission, since administration of the 5-HT reuptake inhibitor fluoxetine or 5-HT_{1A} agonist buspirone increased their sociability (Gould et al., 2011). However, local application of the endocannabinoid agonist anandamide (AEA) or high doses (50–200 mg/kg) of acetaminophen also promoted social interactions in Swiss mice (Umathe et al., 2009). Hence increases in central 5-HT transmission or endocannabinoid levels can promote social behaviors in mice.

Acetaminophen is normally metabolized through sulfation conjugation pathways, but at high doses its metabolic deacetylation products include para-aminophenol and/or N-arachidonoylphenolamine (AM404), an AEA uptake blocker (Beltramo et al., 1997). These metabolites may activate cannabinoid (CB) receptors directly, or indirectly by raising extracellular endogenous CB levels in the brain (Bertolini et al., 2006; Högestätt et al., 2005; Mallet et al., 2008; Ottani et al., 2006). CB agonists such as WIN 55,212-2 can inhibit presynaptic 5-HT release in brain by activating CB_1 receptors found on cell bodies and axons of 5-HT neurons (Lau and Schloss, 2008; Nakazi et al., 2000). Acetaminophen might also be expected to inhibit social behavior via this mechanism, except endocannabinoids have different ligand properties and may be released as mixtures in a region-specific manner, in contrast to systemically-administered agonists.

We aimed to determine if acetaminophen-induced enhancement of Swiss mouse sociability found by Umathe et al. (2009): 1) would occur in socially-deficient strains, and 2) if it is mediated indirectly by AM404, or directly by para-aminophenol action at CB_1 receptors, since its derivatives have some (Ki ~ 200 nM) affinity for them (Sinning et al., 2008). Hence, we examined acetaminophen's effects on social and repetitive behaviors in BTBR and 129S mice, and on endocannabinoid levels in frontal cortex.

Serotonergic tone in the frontal cortex is linked to anxiety and emotional states shaping social behavior (Bartolomucci et al., 2010; Boylan et al., 2007; File and Seth, 2003; Filipenko et al., 2002; Gerretsen et al., 2010). Cannabinoids modulate 5-HT signaling in this region, as exemplified by higher extracellular 5-HT levels in the frontal cortex of CB1 knock-out vs. wild-type mice (Aso et al., 2009). For this reason we measured 5-HT levels after acetaminophen or saline treatment, and compared the density and function of 5-HT_{1A} and CB_1 receptors in the frontal cortex of all strains.

Finally, several human CB_1 receptor gene polymorphisms alter their expression and/or function, and may be associated with social motivation and anxiety disorders (Chakrabarti et al., 2006; Lazary et al., 2009). In mice, CB_1 knock-outs exhibit similar social behavior to wild-types, but not under stressful conditions (Haller et al., 2004). Since CB_1 receptors are intricately involved in brain development, their absence may trigger confounding compensatory alterations in neural function or structure (Hoyle et al., 2011; Trezza et al., 2008). Hence, we compared the sociability of CB_1-deficient (heterozygous) mice to wild-type littermates to determine whether partial loss of CB_1 receptors alone would alter social or repetitive behaviors.

2. Methods

2.1. Mouse subjects

All procedures involving mice were performed in accordance with guidelines for care and use of laboratory animals (National Institutes of Health), and were approved by the institutional animal care and use committee. BTBR T+tf/J, 129S1/SvImJ and C57BL/6 mouse colony founders were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were bred in the animal facilities of the University of Texas Health Science Center through 2 generations. After weaning (postnatal days 23–25), male littermates were housed in groups of 4–5 per cage until behavioral testing at 3–4 months of age. Mice had ad libitum access to food (Teklad, Harlan, Indianapolis, IN) and water in ventilated clear plastic cages lined with chipped wood bedding. The housing room had a 12 h light/dark cycle (lights on/off at 7:00) and was maintained at 20–22 °C.

CB_1 knock-out mice on a C57BL/6 background strain were the product of wild-type × heterozygous crosses. To determine their genotypes, a 0.5–0.75 cm section of tail tip was collected from each mouse. Tail tips were added to a solution of 200 μL DirectPCR (Viagen Biotech, Los Angeles, CA) and 20 μL of 20 μg/μL Proteinase K (Sigma Chemical Co., St. Louis, MO), then incubated at 55 °C until fully digested (5–14 h). To extract DNA, samples were centrifuged and the supernatants were mixed with 500 μL isopropanol. Samples were centrifuged again, the supernatant discarded, and 200 μL of genomic buffer was added to the pellet before storage at −20 °C. PCR amplification was carried out using 1 μL of DNA sample, 6 μL water, 10 μL AccuStart PCR Supermix (Quanta Biosciences, Gaithersburg, MD), and 0.5 μM of each primer G50: 5′-GCTGTCTCTGGTCCTCTTAAA-3′, G51: 5′-GGTGTCACCTCTGAAAACAGA-3′, G54: 5′-CCTACCCGGTAGAATTAGCTT-3′ (Invitrogen, Carlsbad, CA). The PCR was carried out in a Techne TC 3000 thermo cycler (Barloworld Scientific, UK): 4 min at 94 °C, 35 cycles of 45 s at 94 °C, 45 s at 51 °C, 60 s at 72 °C, and concluded with 10 min at 72 °C. Products were run on 1% agarose gel in 1× TBE buffer at 120 V for 2 h. The gel was transferred onto a UV Transilluminator (Spectroline, Westbury, NY) for analysis of band placement. A band at 342 bp signified homozygous knockout; 413 bp signified homozygous wild type; both bands signified a heterozygote.

2.2. Drug administration

Mice were administered acetaminophen (1–400 mg/kg, Sigma) or 0.9% saline solution by intraperitoneal (i.p.) injection. The cannabinoid agonist WIN 55,212-2 (Ascent Scientific, Princeton, NJ) was initially dissolved in dimethyl-sulfoxide (DMSO) and was diluted with saline (1:10) to administer 0.1 mg/kg i.p. in 10% DMSO to mice. A sub-group of controls was treated with 10% DMSO in saline vehicle, they did not differ significantly from saline-treated mice in social and marble burying tests ($F_{1,7} < 1.25$; $p > 0.3$), so these groups were subsequently pooled. Injections were given 30 min prior to introduction into the testing arena, and 50 min prior to testing.

2.3. Behavioral testing and tissue collection

The three-chamber sociability testing procedure for mice was performed as described in Gould et al. (2011). Briefly, pre-conditioning was performed under low red light (16 lx) first for 10 min with the subject confined to the center chamber, then with chamber doors opened so it could explore the whole arena for another 10 min. Just prior to testing, an empty wire cup cage was placed at one end of the arena, and a stranger mouse of the same strain was placed under a cup cage at the opposite end. Stranger mice were preconditioned to cup cage confinement in 3 separate 30 min sessions prior to testing, and were neither litter- nor cage-mates of the subjects. Cup cages were topped with weighted jars (9 cm high × 7 cm diameter) to prevent mice from climbing on top of them. Digital video cameras (Photosmart R742, Hewlett-Packard, Palo Alto, CA) positioned on tripods over the arenas were turned on, the doors were removed and social approach behavior was recorded for 10 min under low red light.