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Sex-specific effects of CB1 receptor antagonism and stress in adolescence on anxiety, corticosterone concentrations, and contextual fear in adulthood in rats

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ABSTRACT

There is a paucity of research regarding the role of endogenous cannabinoid signalling in adolescence on brain and behaviour development. We previously demonstrated effects of repeated CB1 receptor antagonism in adolescence on socioemotional behaviours and neural protein expression 24–48 h after the last drug administration in female rats, with no effect in males. Here we investigate whether greater effects would be manifested after a lengthier delay. In Experiment 1, male and female rats were administered either 1 mg / kg of the CB1 receptor-selective antagonist AM251, vehicle (VEH), or did not receive injections (NoINJ) daily on postnatal days (PND) 30–44 either alone (no adolescent confinement stress; noACS), or in tandem with 1 h ACS. On PND 70, adolescent AM251 exposure reduced anxiety in an elevated plus maze in males, irrespective of ACS, with no effects in females. On PND 73, there were no group differences in either sex in plasma corticosterone concentrations before or after 30 min of restraint stress, although injection stress resulted in higher baseline concentrations in males. Brains were collected on PND 74, with negligible effects of either AM251 or ACS on protein markers of synaptic plasticity and of the endocannabinoid system in the hippocampus and medial prefrontal cortex. In Experiment 2, rats from both sexes were treated with vehicle or AM251 on PND 30–44 and were tested for contextual fear conditioning and extinction in adulthood. AM251 females had greater fear recall than VEH females 24 h after conditioning, with no group differences in within- or between-session fear extinction. There were no group differences in long-term extinction memory, although AM251 females froze more during a re-conditioning trial compared with VEH females. There were no group differences on any of the fear conditioning measures in males. Together, these findings indicate a modest, sex-specific role of CB1 receptor signalling in adolescence on anxiety-like behaviour in males and conditioned fear behaviour in females.

1. Introduction

The contributions of endogenous (i.e., brain derived) cannabinoid signalling in adolescence to the normative development of emotional behaviours and stress reactivity are not well understood. Long-term developmental effects of adolescent exposure to exogenous cannabinoid drugs, however, are well documented in both humans (Crean et al., 2011) and rodents (Lee and Gorzalka, 2015; Rubino and Parolaro, 2008) providing a window to the possible involvement of the ECS in normative adolescent development. Developmental effects of exogenous cannabinoid drugs on brain and behaviour are attributed to actions at the cannabinoid type-1 (CB1) receptor (Renard et al., 2016a). Consistent with effects of cannabinoid drugs on emotional behaviours and neuroendocrine stress responses (McLaughlin and Gobbi, 2012),

Abbreviations: CB1, cannabinoid receptor type-1; PFC, prefrontal cortex; DH, dorsal hippocampus; PND, postnatal day; AM251, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1H-pyrazole-3-carboxamide; CORT, corticosterone; GAD67, glutamic acid decarboxylase isoform 67; PSD95, post-synaptic density protein 95; PKA, protein kinase A; EPM, elevated plus maze; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; GABA, gamma-aminobutyric acid, CP55,940, (-)-cis-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol; THC, Δ^9-tetrahydrocannabinol; Akt, protein kinase B; mTOR, mammalian target of rapamycin; cAMP, cyclic adenosine monophosphate; ERK1/2, extracellular signal-regulated protein kinase-1 and-2

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CB1 receptors are densely expressed in corticolimbic brain regions such as the prefrontal cortex (PFC) and hippocampus (de Fonseca et al., 1993; Tsou et al., 1998, 1999), and there are changes in expression in adolescence. For example, in the PFC, CB1 receptor expression decreased in male rats from PND 29–50 (Ellgren et al., 2008), whereas expression increased in females from PND 46–60, then decreased to adult levels by PND 75 (Rubino et al., 2015). Behaviourally, repeated exposure to CB1 receptor agonists during adolescence is associated with long-term reductions in social behaviours and object recognition memory in rats (O'Shea et al., 2006, 2004; Renard et al., 2013, 2017; Schneider et al., 2008). In accordance with effects on behaviour, adolescent exposure to cannabinoid drugs is associated with long-term alterations to brain structure and function that are evident into adulthood. In male rats, repeated exposure to the CB1 receptor agonist Δ^9-tetrahydrocannabinol (THC) during adolescence induced a state of dopaminergic hyperactivity in the mesocorticolimbic system, as well as reduced the activity of the Akt/mTOR signalling pathway within the PFC when measured in adulthood (Renard et al., 2017). Similarly, male rats exposed to the synthetic CB1 receptor agonist CP55,940 in adolescence were found to have reduced PFC dendritic complexity, reduced hippocampal-induced PFC plasticity, and reductions in the glutamatergic synaptic marker PSD95 in the PFC in adulthood (Renard et al., 2016b). In female rats repeatedly exposed to THC during adolescence there was a similar reduction in dendritic complexity within the PFC, however, the effects on markers of glutamatergic synapses were opposite to those previously reported in males; THC treated females had increased expression relative to vehicle-treated females (Rubino et al., 2015). That disruptions to normative CB1 receptor signalling via administration of agonists leads to altered neuronal and behavioural phenotypes is not surprising given the role of CB1 receptors in regulating trans-synaptic communication (Viveros et al., 2007). Nevertheless, the role of endogenous cannabinoid signalling during adolescence on brain and behaviour development is poorly understood.

Under normative physiological conditions, CB1 receptors are activated by a family of lipid neuromodulators known as endocannabinoids (Di Marzo, 2011). Although endogenous cannabinoid signalling in adolescence has not been well characterized, studies investigating the effects of repeated administration of the CB1 receptor selective antagonist / inverse agonist AM251 during adolescence have confirmed a role for endocannabinoid signalling during this period of development. In male rats, adolescent AM251 exposure (5 mg / kg, PND 35–45) resulted in increases in risk-assessment and active stress-coping behaviours (as measured by increased time spent struggling in a forced swim test), as well as increases in PFC CB1 receptor expression, when tested several weeks after the final drug exposure (Lee et al., 2015). In female rats, repeated AM251 exposure during adolescence (0.5 mg / kg, PND 35–45) prevented developmental decreases in glutamatergic markers within the PFC into adulthood (Rubino et al., 2015). Previously, we reported sex-specific effects of repeated adolescent CB1 receptor antagonism (1 mg / kg, PND 30–44) on brain and behaviour when tested 24–48 h after the final exposure to the antagonist, and drug effects were present whether exposure occurred alone or in tandem with a psychological stressor (Simone et al., 2018). Specifically, AM251 increased time spent in social interactions, increased the expression of the PFC GABAergic marker GAD67, and reduced dorsal hippocampal CB1 receptor expression in female, but not in male, rats (Simone et al., 2018). Nevertheless, many manipulations in adolescence require an incubation period before their effects are manifested (Isgor et al., 2004; Lee et al., 2015; Trauth et al., 2000). Thus, in experiment 1, we tested the hypotheses that (1) repeated adolescent CB1 receptor antagonism will alter neurodevelopment leading to changes in anxiety-like behaviour and neuroendocrine stress responses in adulthood, and that (2) effects would be more pronounced when antagonism occurred in tandem with a stressor than when alone because of the on-demand nature of endocannabinoid signalling (i.e., an increase in endocannabinoid release is expected in response to stress (Hillard, 2014)). To address these questions, male (experiment 1 A) and female (experiment 1B) rats were treated with either the CB1 receptor selective antagonist / inverse agonist AM251 or vehicle, or were untreated, on PND 30–44. Males and females were investigated in separate experiments because of the high number of comparisons within each sex, and because it is expected that any effects observed would be sex-specific. To investigate endocannabinoid-stress interactions, half of all rats from each experiment received treatment and were immediately placed back into their homecages, whereas the other half received treatment immediately before 1 h of confinement stress. Measures of anxiety (elevated plus maze), neuroendocrine stress responses (plasma corticosterone), and neural protein markers of the endocannabinoid system (CB1 receptors, PKA), GABAergic system (GAD67), glutamatergic system (PSD95), as well as a marker for synaptic plasticity (spinophilin), were obtained in adulthood (PND 70–74) several weeks after the final exposure to AM251. In a second experiment, we investigated the long-term effects of adolescent AM251 on conditioned fear behaviours in male (experiment 2 A) and female (experiment 2B) rats. We previously reported AM251-mediated increases in PFC GAD67 expression and decreases in dorsal hippocampal CB1 receptor expression (Simone et al., 2018), thus we tested rats in a contextual fear conditioning and extinction paradigm; contextual fear conditioning and extinction have been shown to critically depend on dorsal hippocampal and PFC signalling (Maren, 2001; Quirk and Mueller, 2008; Sierra-Mercado et al., 2011). Because the behavioural effects of AM251 observed in experiment 1 were independent of stress exposures, we limited our comparisons in experiment 2 to vehicle and AM251 rats only, allowing for an increase in statistical power and a reduced number of rats.

2. Methods

2.1. Animals

Ninety-six Long-Evans rats for experiment 1 (48 male and 48 female) and 48 Long-Evans rats for experiment 2 (24 male and 24 female) were obtained from Charles River (Kingston, New York, USA) and arrived at the Brock University Comparative Bioscience Facility on PND 25. Rats were housed in same-sex pairs and kept on a 12-hour light-dark cycle (lights on at 8:00 h) with *ad libitum* access to food and water. All procedures were approved by the Brock University Institutional Animal Care Committee and were in accordance with the Canadian Council on Animal Care and with the National Institutes of Health guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs and injections

For both experiments 1 and 2, the CB1 receptor antagonist / inverse agonist AM251 (Cayman Chemical, USA) was dissolved in a 1:1:18 dilution of DMSO, Tween-80, and 0.9% saline and administered i.p. at a dose of 1.0 mg / kg. The vehicle was a 1:1:18 mixture of DMSO, Tween-80, and 0.9% saline in the absence of AM251. The dose of AM251 was chosen based on previous work from our lab (Simone et al., 2018). Injections were administered at a volume of 1.0 ml/kg. For experiment 1, vehicle (VEH) and AM251 injected rats in the noACS groups were returned directly to their homecages after injection. VEH and AM251 injected rats in the ACS groups were injected immediately before the confinement stress procedure each day. To account for any stress of injection effects we also included a no injection (NoINJ) comparison group for both the noACS and ACS conditions.

2.3. Experiment 1: long-term effects of AM251 and stress exposures in adolescence

See Fig. 1 for the experimental design and timeline. Testing