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CANNABINOID RECEPTOR TYPE 1 ANTAGONISM SIGNIFICANTLY MODULATES BASAL AND LOUD NOISE INDUCED NEURAL AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS RESPONSES IN MALE SPRAGUE–DAWLEY RATS

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Abstract—Altered regulation of the hypothalamic-pituitary-adrenal (HPA) axis is associated with stress-induced changes in cognitive, emotional, and physical health. Recent evidence indicates that the endogenous cannabinoid (eCB) system may modulate HPA-axis function both directly and more centrally, via regulation of limbic brain systems that control HPA-axis activity. The current study examines the contribution of cannabinoid type 1 (CB1) receptor modulation throughout the neuraxis on control and stress-induced HPA-axis activity. Adult male Sprague–Dawley rats were given intraperitoneal injections of either CB1 receptor antagonist (AM251, 2 mg/kg) or vehicle 30 min prior to a session of loud white noise stress (95 dBA for 30 min) or placement in a familiar sound-proof chamber. Immediately following stress and control treatments, rats were killed, the brains and pituitary glands were excised for subsequent immediate early gene (*c-fos* mRNA) measurement, and trunk blood was collected for subsequent determination of corticosterone (CORT) and adrenocorticotropic (ACTH) hormone levels. AM251 treatment resulted in a potentiated plasma ACTH response to loud noise stress. AM251 treatment also increased stress-induced plasma CORT levels, but that increase may be due to an increase in basal plasma CORT levels, as was evident in control rats. AM251 treatment produced three distinctive *c-fos* mRNA response patterns across the various brain regions examined. In cortical (prelimbic, infralimbic, somatosensory, and auditory) and some subcortical structures (basolateral amygdala and paraventricular nucleus of the hypothalamus), AM251 treatment produced a substantial increase in *c-fos* mRNA that was comparable with the elevated *c-fos* mRNA levels present in those brain regions of both vehicle and AM251-treated stressed rats. In some other subcortical structures (bed nucleus of the stria terminalis and medial preoptic area) and the anterior pituitary, AM251 treatment produced a *c-fos* mRNA response pattern that was similar to the response pattern of ACTH hormone levels, that is, no effect on no noise control levels, but an augmentation of stress-induced levels. Conversely, in the medial geniculate and ventral posterior thalamus, AM251 treatment inhibited stress-induced *c-fos* mRNA induction. These data indicate that disruption of eCB signaling through CB1 receptors results in potentiated neural and endocrine responses to loud noise stress, but also substantial increases in activity in various brain regions and the adrenal gland.

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Key words: endocannabinoid, HPA axis, AM251, stress, noise.

Accumulating evidence implicates the endogenous cannabinoid (eCB) system as an important regulator of emotionality and stress reactivity (Finn, 2010; Gorzalka and Hill, 2009; Hill et al., 2010; Lutz, 2009; Reibe and Wotjak, 2011; Valverde, 2005). eCB modulate central nervous system activity through two established ligands, anandamide (Devane et al., 1992) and 2-arachidonylglycerol (2AG) (Sugiura et al., 1995), which are rapidly synthesized by specific enzymes in postsynaptic neurons in response to calcium-dependent synaptic signaling or other metabotropic receptor activation (Stella and Piomelli, 2001). Once produced, the highly lipid-soluble eCB interact with presynaptic eCB receptors and downstream second-messenger cascades, where they generally have been shown to inhibit glutamate, GABA, acetylcholine, norepinephrine, and serotonin release, among others (Freund et al., 2003; Schlicker and Kathmann, 2001). Both eCB ligands bind to type 1 cannabinoid receptors (CB1), which are the most widely expressed eCB receptors in the central nervous system (Herkenham et al., 1991), and to the centrally more restricted type 2 receptors (CB2) (Atwood and Mackie, 2010). In addition to their central nervous system actions, eCB also have well characterized peripheral actions through the same receptor subtypes (Atwood and Mackie, 2010). Given the widespread influence of eCB on neurotransmission, the overall contribution of eCB activity on specific neural functions has been difficult to precisely define.

Recent studies suggest that the endogenous cannabinoids negatively regulate stress responsiveness (Cota, 2008; Hill and McEwen, 2010; Hill et al., 2009; Patel et al., 2004, 2005; Tasker, 2004). For instance, genetic deletion of CB1 receptors in mice results in hyperactive hypothalamo-pituitary-adrenal (HPA)-axis responses to a variety of laboratory stressors, as indexed by increased

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Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CB1, cannabinoid type 1; CORT, corticosterone; dB, decibel; DC, drug-treated non-stressed control; DMSO, dimethyl sulfoxide; DN, drug-treated noise-stressed; eCB, endocannabinoid; HPA, hypothalamus-pituitary-adrenal; MPA, medial preoptic area of the hypothalamus; PVN, paraventricular hypothalamic nucleus; SSC, saline sodium citrate; VC, vehicle-treated non-stressed control; VN, vehicle-treated noise-stressed; VPL, ventral posterior lateral thalamus; VPM, ventral posterior medial thalamus.

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plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels (Aso et al., 2008; Cota et al., 2007; Haller et al., 2004; Steiner et al., 2008; Uriguen et al., 2004). CB1 receptor knockout mice also express a heightened circadian peak of plasma CORT and impaired glucocorticoid negative feedback compared with wild-type mice (Cota et al., 2007). Furthermore, systemic pharmacological antagonism of CB1 receptors in mice potentiates CORT release in response to restraint and forced swim stress (Patel et al., 2004; Steiner et al., 2008) as well as stress-induced neuronal activity (as indexed by Fos) in the paraventricular nucleus of the hypothalamus (Patel et al., 2004), cingulate cortex, lateral septum, and nucleus accumbens (Patel et al., 2005). On the other hand, increasing the availability of anandamide by systemic pharmacologic blockade of the enzyme responsible for its degradation results in reduced CORT release to restraint stress (Patel et al., 2004, 2005).

Much of the reported work examining the role of eCB activity in stress and HPA-axis regulation has been performed using mice, though rats have been used in examining the specific involvement of the basolateral amygdala (Hill et al., 2009) and hypothalamic nuclei (Di et al., 2003, 2005a,b, 2009; Evanson et al., 2010; Ginsberg et al., 2010). Increases in regional Fos protein expression in multiple limbic regions were observed in mice after administration of a CB1 receptor antagonist followed by restraint challenge, suggesting that the stress modulatory effects of eCB are not limited to the HPA axis (Patel et al., 2005). To our knowledge, no published work has examined the effects of acute stress and CB1 receptor antagonism on c-fos mRNA expression in rats. Differences in reported patterns of stressor-induced limbic eCB levels in mice (Patel et al., 2004, 2005; Rademacher et al., 2008) compared with rats (Hill et al., 2009) suggest possible species differences that warrant further investigation of the functional contribution of eCB processes in stress responses in rats. In addition, no studies have explored the possibility that CB1 receptor antagonism modifies sensory processing that might then be reflected in limbic and hypothalamic structures, in response to stress (Patel et al., 2005). The current study was therefore undertaken to assess regional c-fos mRNA induction in several sensory, limbic, and hypothalamic nuclei, as well as pituitary (ACTH) and adrenal (CORT) hormone responses to CB1 receptor antagonism on control and acute loud noise exposure. We chose to examine c-fos mRNA in contrast to Fos protein because of the rapid induction and transient expression of mRNA after the onset of neuronal signaling. c-fos mRNA likely provides a tighter temporal representation than Fos protein of relative neural activity to proximal events immediately preceding brain harvesting.

EXPERIMENTAL PROCEDURES

Subjects

Forty-two male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing 275–300 g upon arrival were used. Animals were housed in polycarbonate tubs containing wood shavings, with wire lids providing rat chow and water *ad libitum*. Conditions in the animal colony were controlled to constant humidity and temperature, with a 12:12-h light/dark cycle (lights on at 7:00 AM). Testing was performed between 8:30 AM and 12:30 PM during the circadian nadir for the HPA axis. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Colorado and conformed to the United States of America National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and the number of animals used.

Acclimation

Animals were allowed two weeks of acclimation to the colony before testing. The first week, animals were housed in groups of four. During the second week of acclimation, rats were individually housed and handled daily, in the colony room, from days one through four. On each of the last three days before testing, rats were transported in their home cages from the colony to the testing room, handled, returned to their home cages, and placed inside individual acoustic chambers (without noise exposure) for thirty minutes. This pre-exposure was intended to familiarize the rats to all of the testing procedures and minimize novelty-related responses on the test day.

Drug treatment

Rats were randomly assigned to one of four groups: Vehicle treated and noise exposed ($n=10$), Vehicle-treated controls ($n=10$), AM251 treated and noise exposed ($n=12$), and AM251-treated controls ($n=10$), in a 2×2 balanced design. The CB1 antagonist/inverse agonist AM251 (Ascent Scientific, Princeton, NJ, USA) was used to assess the involvement of the endogenous cannabinoid system on control and acute loud noise exposure. AM251 was dissolved in dimethyl sulfoxide (DMSO), Tween 80, and physiological (0.9%) saline (in a 1:1:8 ratio, respectively). We experienced difficulty in keeping AM251 from precipitating out of solution, so on the testing day, a stir plate was used to maintain suspension, and syringes were loaded immediately prior to dosing. AM-251-treated rats received a single intraperitoneal injection of 2 mg/kg, in injection volumes of 1 ml/kg. This dosage was chosen based on pilot testing in our laboratory suggesting that this dose was adequate to produce enhancement of loud noise-induced HPA-axis activity. On the test day, rats received a single intraperitoneal injection of AM251 or a similar volume of vehicle (DMSO/Tween 80/0.9% saline) 30 min prior to placement in the acoustic chambers.

Loud noise procedures

The acoustic chambers used in this experiment have been described in detail in Day et al. (2009). On the testing day, rats were placed in the acoustic chambers in their home cages 30 min after vehicle or AM251 injection. Rats were either kept under quiet "no noise" control conditions (background noise of fans approximately 57 dB SPL—A scale) or loud noise (95 dB) was turned on immediately and remained on for 30 min. Immediately upon noise termination or quiet chamber exposure, rats were removed from the acoustic chambers, sacrificed by decapitation, and trunk blood was collected in chilled EDTA-containing Vacutainer tubes. Brains and pituitary glands were immediately harvested and frozen in −30 to −40 °C isopentanes.

Corticosterone enzyme-linked immunosorbent assays (ELISA)

The corticosterone assay was performed according to the manufacturer's instructions (AssayDesigns, Ann Arbor, MI, USA) with one modification. Ten microliters of plasma in the standard buffer