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Modulation of OCT3 expression by stress, and antidepressant-like activity of decynium-22 in an animal model of depression

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ABSTRACT

The organic cation transporter-3 (OCT3) is a glucocorticoid-sensitive uptake mechanism that has been shown to regulate the bioavailability of monoamines in brain regions that are implicated in the pathophysiology of depression. In the present study, the relative impacts of acute stress alone and acute stress with a history of repeated stress (chronic + acute) were evaluated in two strains of rats: the stress-vulnerable Wistar–Kyoto (WKY) strain and the somewhat more stress-resilient Long–Evans (LE) strain. OCT3 mRNA was significantly upregulated in the hippocampus of LE rats 2 h after exposure to acute restraint stress, but not in acutely-restrained rats with a history of repeated social defeat stress. WKY rats exhibited a very different pattern. OCT3 mRNA was unaffected by acute restraint stress alone but was robustly upregulated after repeated + acute stress. There was also a corresponding increase in cytosolic OCT3 protein following repeated + acute stress in WKY rats 3 h after presentation of the acute stressor. These results are consistent with the hypothesis that altered expression of the OCT3 may play a role in stress coping, and strain differences in regulation of this expression may contribute to differences in physiological and behavioral responses to stress. Furthermore, the OCT3 inhibitor, decynium 22 (1 and 10 μg/kg, i.p.) reduced immobility of WKY rats, but not that of LE rats, in the forced swim test, suggesting that blockade of the OCT3 has antidepressant-like effects. Since WKY rats also appear to be resistant to the behavioral effects of traditional antidepressants, this also suggests that OCT3 antagonism may be an alternative therapeutic strategy for the treatment of depression in individuals who do not respond to conventional antidepressants.

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1. Introduction

Major depressive disorder has been described as a disorder of stress regulation that can impact monoaminergic systems in the brain, producing profound alterations in mood and emotional behavior (for a review see Hamon and Blier, 2013). Indeed, elevated activity of the hypothalamic–pituitary–adrenocortical (HPA) axis is implicated in the pathophysiology of depression, and many of the antidepressant drugs that specifically modify serotonergic and/or adrenergic tone concurrently restore normal HPA axis functioning (Schüle, 2007).

It is widely recognized that some individuals are more susceptible to depression than others. The biochemical basis of these individual differences is not fully characterized, but evidence is accruing that reciprocal interactions between stress responses and monoaminergic neurotransmission play key roles in the pathogenesis of depression. In agreement with this perspective, there is evidence that polymorphisms in genes that regulate the bioavailability or post-synaptic effects of serotonin may contribute to individual differences in vulnerability. For instance, carriers of the s allele of the SLC6A4 gene that encodes the serotonin transporter (SERT) are at increased risk for depression (Caspi et al., 2003). These individuals have dysregulated HPA axis function, resulting in elevated waking cortisol and adrenocorticotropic hormone (ACTH) levels (Chen et al., 2009; Wüst et al., 2009).

Alterations in noradrenergic neurotransmission also appear to contribute to the pathophysiology of depression. Norepinephrine (NE) is released by terminals of neurons that originate in the locus coeruleus, a nucleus that is thought to be sensitized in major depression, and normalized by antidepressant treatment (Chandley et al., 2014). Acute treatment with various antidepressant medications each reduce spontaneous and sensory-evoked firing of LC neurons (Grant and Weiss, 2001; West et al., 2009), and chronic treatment with these drugs restores extra-neuronal concentrations of NE in cortical regions that are innervated by LC projections (Linner et al., 1999; Matteo et al., 2001). Thus, antidepressant drugs may potentially reverse the pathology of depression (at least in part) through noradrenergic re-regulation (Grant and Weiss, 2001).

The organic cation transporter-3 (OCT3) represents a potential link between monoaminergic function and stress neurobiology that may help modulate vulnerability for, and recovery from major depression. This transporter belongs to a family of polyspecific solute carriers that

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increase the elimination of cationic species (Pritchard and Miller, 1993; Ullrich, 1994), including monoamines from the extracellular space (Gründemann et al., 1998, 1999; Schömig et al., 2006). Whereas the OCT1 and OCT2 subtypes are predominantly expressed in visceral organs, OCT3 mRNA is abundantly expressed within the brain, especially in cerebellum, hippocampus, pontine nuclei and cortex (Kekuda et al., 1998; Wu et al., 1998). In these regions, it is distributed along monoaminergic pathways, where it mediates a low-affinity, high-capacity, glucocorticoid-sensitive system for removing extracellular monoamines (Amphoux et al., 2006; Gasser et al., 2006, 2009).

Overall, it appears that the low-affinity, high-capacity function of the OCT3 transport system may be especially salient when the classical high-affinity monoamine transporters are saturated or in low abundance. This was demonstrated in mice with a homozygous deletion of the serotonin transporter (SERT). These interesting knockouts exhibit an abnormal phenotype that includes elevated anxiety-related behavior and exaggerated stress responses (Holmes et al., 2003). However, these mice maintain hippocampal 5HT levels at relatively normal concentrations due to upregulation of the OCT3 in the hippocampus (Schmitt et al., 2003; Baganz et al., 2008). Since corticosteroids and stress provoke rapid increases in extracellular monoamine concentrations (Lowry et al., 2001, 2003), we propose that stressors may provide the necessary conditions for the OCT3 to exert a physiologically meaningful effect on monoaminergic neurotransmission.

The first goal of this study was to examine OCT3 expression after stress in Wistar–Kyoto (WKY) and Long–Evans (LE) rat strains, focusing primarily on the hippocampus, medial prefrontal cortex, and striatum, where monoaminergic and stress-responsive systems converge. The WKY rat exhibits relatively greater behavioral and neuroendocrine responses to stress, which may indicate a heightened sensitivity to the effects of corticosterone (Paré and Redei, 1993; Tejani-Butt et al., 1994; Bielajew et al., 2002; Rittenhouse et al., 2002). Furthermore, brain 5HT systems appear to be dysregulated in WKY rats after stress compared to responses in other strains (Paré and Tejani-Butt, 1996; De La Garza and Mahoney, 2004). A recent study suggests that stress, corticosterone sensitivity, and OCT3 expression levels are positively correlated (Baganz et al., 2010). Accordingly, we hypothesized that OCT3 expression would be upregulated more in the stress-sensitive WKY rat than in the LE rat. We also examined hippocampal expression of the glucocorticoid receptor (GR) and SERT, both of which exhibit plasticity in animal models of stress and depression (Paré and Tejani-Butt, 1996; Marini et al., 2006; Wu et al., 2011).

In a second experiment, we assessed the effects of decynium 22 (D22; a cyanine dye derivative that inhibits OCT3 transport) on WKY and LE rats in the forced swim test. Previous studies revealed that D22 reduced immobility in the tail suspension test (an antidepressant-like effect) in SERT knockout mice while failing to alter the behavior of wild type conspecifics (Baganz et al., 2008). Furthermore, microdialysis studies have shown that intracranial administration of D22 into the dorsomedial hypothalamus (a region implicated in endocrine, autonomic, and behavioral responses to stress) elicits robust increases in extracellular 5HT concentrations (Feng et al., 2005), providing a potential neurochemical mechanism that could contribute to an antidepressant-like effect. In accordance with these observations, we hypothesized that D22 would exert an antidepressant-like effect selectively in the stress-sensitive WKY strain.

2. Materials and methods

2.1. Experiment 1 — effect of stress on OCT3 gene and protein expression

All experimental protocols were pre-approved by the University of Florida Institutional Animal Care and Use Committee (IACUC) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2003).

2.1.1. Animals and housing

Male LE and WKY rats (250–300 g; n = 48 per strain) were pair-housed in standard polycarbonate cages (43 × 21·5 × 25.5 cm) in a climate controlled vivarium with a 12-h light/dark cycle (lights on at 7 am) and were given 7 days to adapt to the AAALAC-approved housing facility before the experiments began. The rats were allowed *ad libitum* access to standard laboratory chow (Lab Diet 5001) and tap water. These rats were randomly assigned to control, acute restraint stress, or repeated social defeat (*i.e.*, *intruder* rats) plus acute restraint stress groups (n = 8 per group). An additional 8 male LE rats (*i.e.*, *resident* rats; 600–650 g) were singly-housed in standard polycarbonate cages and were trained to exhibit dominant home-cage behaviors in the social defeat paradigm. An additional 4 male LE rats (*i.e.*, *training* rats; 250–300 g) were used to train the dominant residents.

2.1.2. Dominant resident training

During each daily dominance-training session, a training rat was placed inside the resident's home cage for up to 5 min. If the training rat was defeated 3 times (*i.e.*, maintained a supine posture for a minimum of 2 s), the resident was classified as dominant for that session. Once dominance was established, the test session was terminated. This procedure was repeated daily (up to 30 days) until there were 3 consecutive days in which the resident defeated the training rat 3 times per session. Resident rats were used in social defeat sessions only after they passed this training.

2.1.3. Experimental design

Each of the rats in the acute stress groups was handled once daily on each of seven consecutive days, exposed to a single 30 min session of restraint stress on day 8, and was killed 120 min (n = 24 rats per strain) or 180 min (n = 24 rats per strain) after the beginning of the restraint session. Each of the intruder rats in the chronic + acute stress groups was exposed to one daily social defeat session on each of 7 consecutive days. On day 8, these rats were exposed to 30 min of restraint stress, and were killed 120 min (n = 24 rats per strain) or 180 min (n = 24 rats per strain) after the beginning of the restraint session. The control rats were handled once daily but were otherwise undisturbed, and were killed at the same time as the other rats on day 8 (n = 8 LE and 8 WKY rats in each of the control, acute stress, and chronic + acute stress conditions).

2.1.4. Social defeat

The social defeat procedure is based on previous methods described in detail in Marcinkiewcz et al. (2009). Briefly, this procedure consisted of two stages. In the first stage, the intruder LE or WKY rat was placed into a 10 × 10 × 15 cm (inner dimensions) double-walled wire mesh protective cage. The rat was then placed, inside the protective cage, into the home cage of the resident rat for 45 min. The cage served to separate the rats, avoiding physical contact, but maintaining stressful sensory stimuli. During the second stage, the intruder rat was removed from the wire mesh cage and placed directly inside the resident's home cage. Dominance/submission behavior was scored as described for the training sessions. This direct interaction between resident and intruder rats continued for a maximum of 5 min, or until 3 defeats occurred. Every intruder rat was exposed to a different resident rat in each of the 7 defeat sessions. After each exposure to a resident rat, the intruder rat was removed and returned to its home cage.

2.1.5. Restraint stress

Restraint stress was performed according to the methods that we have previously described (Devine et al., 2003; Simpkiss and Devine, 2003; King et al., 2007). Briefly, each rat was individually removed from its home cage and placed into a restraining tube for 30 min. The restraint tube was composed of a flexible sheet of plastic (11″ × 7 3/4″) mounted to a rigid Plexiglas cradle (8 1/2″ × 3′ × 3″), with a vertical slot to allow comfortable placement of the tail, and ventilation holes to allow unrestricted breathing. The plastic sheet was gently rolled