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Deletion of Rictor in catecholaminergic neurons alters locomotor activity and ingestive behavior

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ABSTRACT

While the etiology of depression is not fully understood, increasing evidence from animal models suggests a role for the ventral tegmental area (VTA) in pathogenesis. In this paper, we investigate the potential role of VTA mechanistic target of rapamycin 2 (TORC2) signaling in mediating susceptibility to chronic social defeat stress (CSDS), a well-established mouse model of depression. Utilizing genetic and viral knockout of Rictor (rapamycin-insensitive companion of target of rapamycin), a requisite component of TORC2, we demonstrate that decreasing Rictor-dependent TORC2 signaling in catecholaminergic neurons, or within the VTA specifically, does not alter susceptibility to CSDS. Opiate abuse and mood disorders are often comorbid, and previous data demonstrate a role for VTA TORC2 in mediating opiate reward. Thus, we also investigated its potential role in mediating changes in opiate reward following CSDS. Catecholaminergic deletion of Rictor increases water, sucrose, and morphine intake but not preference in a two-bottle choice assay in stress-naïve mice, and these effects are maintained after stress. VTA-specific knockout of Rictor increases water and sucrose intake after physical CSDS, but does not alter consummatory behavior in the absence of stress. These findings suggest a novel role for TORC2 in mediating stress-induced changes in consummatory behaviors that may contribute to some aspects of mood disorders.

1. Introduction

Depression is a serious mental illness that induces a significant societal burden as a leading cause of disability (Ferrari et al., 2013) and is highly co-morbid with other disorders such as drug addiction (Swendsen and Merikangas, 2000; Volkow, 2004). While the exact causes of depression remain elusive, a combination of factors, including stressful life events, are known to increase the likelihood of developing a major mood disorder (Shapero et al., 2014). Increasing evidence suggests a role for the dopamine reward circuit, and specifically activity of ventral tegmental area (VTA) dopamine neurons, in mediating susceptibility to chronic social defeat stress (CSDS), a rodent model of depression (Krishnan et al., 2007). Moreover, CSDS also induces biochemical changes in the VTA, including decreased phosphorylation of AKT at Ser473 (pAKT), and preventing or mimicking this biochemical event is sufficient to rescue or induce CSDS susceptibility, suggesting changes in VTA AKT activity are behaviorally relevant (Krishnan et al., 2008). Interestingly, VTA pAKT is also decreased in rats and mice treated chronically with morphine, and modulation of VTA AKT activity is sufficient to alter opiate reward, as measured by conditioned place preference (CPP) (Russo et al., 2007). Together, these data suggest that alteration of VTA AKT phosphorylation plays a critical role in both mood disorders and drug reward.

AKT is phosphorylated at Ser473 by the mechanistic target of rapamycin complex 2 (TORC2) (Sarbassov et al., 2005), and we have recently shown that altering TORC2 activity in the VTA is sufficient to induce changes in morphine reward (Mazei-Robison et al., 2011). Given the lack of a selective pharmacological inhibitor, we altered VTA TORC2 signaling via genetic deletion and viral-mediated overexpression of rapamycin-insensitive companion of TOR (Rictor), as this protein is a necessary component for TORC2 kinase

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activity. Global deletion of TORC2 is embryonically lethal (Shiota et al., 2006), and thus floxed-Rictor mice have been developed and used in combination with Cre driver lines (Dadalko et al., 2015a, 2015b; Siuta et al., 2010; Thomanetz et al., 2013) or stereotaxic infusion of AAV-Cre in adult mice (Mazei-Robison et al., 2011) to produce cell-type or brain-region specific Rictor KO mice to allow examination of the role of TORC2 signaling *in vivo*. Recently, floxed-Rictor mice have been crossed to the tyrosine hydroxylase (TH)-Cre reporter line to KO TORC2 signaling specifically from catecholaminergic neurons (TH-Rictor) (Dadalko et al., 2015a). TH-Rictor-KO mice display an increase in novelty-induced locomotion compared to their wild-type controls, as well as an increase in lean and overall body mass, but with no reported difference in fat mass (Dadalko et al., 2015a).

Given our data that VTA KO of Rictor was sufficient to modulate morphine reward, we sought to determine whether KO of Rictor in the VTA or in TH neurons would increase susceptibility to CSDS, as predicted by pAKT results. Further, we sought to determine whether VTA- or TH-Rictor-KO mice would have altered morphine reward following CSDS. While data on morphine reward following CSDS is limited (Covington and Miczek, 2001; Vivian and Miczek, 1999), susceptible mice have increased cocaine CPP following CSDS (Krishnan et al., 2007) and social defeat stress also increases cocaine self-administration in both rats (Covington and Miczek, 2001) and mice (Han et al., 2015). Thus, we assessed voluntary intake and preference for morphine using a two-bottle choice test. We found that while decreasing TORC2 signaling in either TH neurons or in the VTA does not increase susceptibility to CSDS, there were changes in consummatory behavior between Rictor KO mice and controls. Interestingly, whereas there were differences between TH- and VTA-Rictor KO prior to CSDS, all Rictor KO mice exhibited a similar phenotype post-CSDS. These data suggest that additional TORC2 substrates may exhibit competing effects to those of AKT in stress susceptibility and that behavioral outputs of TORC2 signaling may be dependent on subsets of catecholaminergic neurons.

2. Materials and methods

2.1. Mice

All mice were housed at 22–25 °C on a 12 h light/dark cycle with food and water available *ad libitum*. Experiments utilized adult male and female mice (8–15 weeks). Homozygous floxed Rictor mice were generated as previously described (Mazei-Robison et al., 2011; Shiota et al., 2006; Siuta et al., 2010), and were also crossed with heterozygous tyrosine hydroxylase (TH)-Cre mice (Jackson Laboratories, 008601) to generate developmental Rictor knock-out (KO) mice (Dadalko et al., 2015a); all mice were fully backcrossed to the c57Bl/6 background. Mouse genotypes were verified at 21–28 days using standard procedures. Published primers to assess floxed-Rictor (5′- CCT GAG CAG TGC CCG ACT TCT CTA G-3′ and 5′-CCT TTC GCA TCG CCA CTG CA-3′) and TH-Cre (5′- GAT CTC CGG TAT TGA AAC TCC AGC-3′ and 5′- GCT AAA CAT GCT TCA TCG TCG G-3′) status were used. Of note, Cre-mediated deletion of Rictor using this floxed-Rictor line (via either AAV-Cre infusion or cross with Cre-driver line) has been shown to be sufficient to disrupt TORC2-mediated kinase activity as assessed by phosphorylation of AKT at Ser473 (Dadalko et al., 2015b; Mazei-Robison et al., 2011; Siuta et al., 2010). For social defeat stress studies, retired CD-1 male breeders (Charles River) were purchased and screened for aggressive behavior as described previously (Golden et al., 2011). All experiments were approved by the Institutional Animal Care and Use Committee at Michigan State University.

2.2. Drugs

Morphine sulfate (generously provided by the NIDA Drug Supply Program) and quinine sulfate (Sigma) were dissolved in water for drinking studies.

2.3. Viral-mediated gene transfer

Stereotaxic surgeries were completed as previously described (Mazei-Robison et al., 2011). Briefly, mice were anesthetized (100 mg/kg ketamine, 10 mg/kg xylazine) and bilateral infusions (0.5 μl) of AAV-GFP or AAV-Cre-GFP (UNC Vector Core) were targeted to the VTA (from bregma: −3.2 mm AP, +1.0 mm ML, and −4.6 mm DV, 7° angle). Mice were allowed to recover for ≥14 days prior to behavioral testing to allow for Cre-mediated gene silencing and the degradation of all remaining Rictor in target cells.

2.4. Validation of Rictor deletion

2.4.1. Viral targeting

Following experimental testing, mice were perfused with 4% paraformaldehyde-PBS and brains were cryo-preserved in 30% sucrose-PBS. Brains were sectioned (30 μm) and bilateral VTA targeting was confirmed by GFP expression. The representative VTA targeting and viral expression shown in Fig. 4A was generated using standard immunohistochemistry techniques to label GFP- (Life Technologies A11122, 1:3000) and TH-positive (Sigma, T1299, 1:3000) cells in the VTA (Mazei-Robison et al., 2011). Mice with GFP expression outside the VTA were not included in analyses.

2.4.2. Quantitative Real-Time (RT)-PCR

Rictor deletion was verified by RT-PCR using published procedures (Mazei-Robison et al., 2011). Briefly, VTA was microdissected from mice and stored at −80 °C until processing. RNA was isolated and purified from VTA using Trizol and RNeasy microcolumns (Qiagen). Following reverse-transcription (Applied Biosystems), RNA levels were quantified by RT-PCR using the ΔΔCt method and GAPDH as a normalization control, and all analyses were performed in triplicate. All primers were previously validated: Rictor: 5′-ATG GCG GCG ATC GGC CGC G-3′ and 5′-GAT ACT CCT TGC AAT TTG GCC ACA-3’; GAPDH: 5′- AGG TCG GTG TGA ACG GAT TTG-3′ and 5′- TGT AGA CCA TGT AGT TGA GGT CA-3’; Cre: 5′-CCC GGC AAA ACA GGT AGT TA-3′ and 5′- GAA CGA AAA CGC TGG TTA GC-3’ (Berton et al., 2006; Mazei-Robison et al., 2011).

2.5. Chronic social defeat stress (CSDS)

CSDS was performed as previously described (Golden et al., 2011). Briefly, male control and Rictor KO mice were subjected to a brief daily physical encounter in the home-cage of an aggressive CD-1 retired breeder followed by sensory contact for the following 24 h via a perforated plexiglass partition. Non-stress controls were handled and housed across from a novel c57Bl6 mouse daily. Following the 10th defeat episode, mice were singly housed. A variant of CSDS that utilizes a purely psychological stressor, witness or emotional stress, was also performed as previously described (Warren et al., 2013). Emotional CSDS was performed as described above, with the exception that a second experimental mouse was placed on the opposite side of the plexiglass partition during the physical encounter allowing this mouse to witness physical social defeat stress.

2.6. Behavioral overview

Behavioral characterization was completed ≥14 days following