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Paradoxical effects of the endocannabinoid uptake inhibitor VDM11 on accumbal neural encoding of reward predictive cues

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Abstract

A growing body of evidence implicates the endocannabinoid (eCB) system in brain reward function. Previous studies show that antagonizing eCB transmission decreases reward-directed behavior and nucleus accumbens (NAc) encoding of reward predictive cues. We therefore hypothesized that elevating eCB levels would uniformly facilitate NAc neural encoding of reward predictive cues and reward-directed behavior. Contrary to our expectations, the eCB transport uptake inhibitor, VDM11, dose-dependently decreased both measures.

Keywords

endocannabinoid uptake; medium spiny neuron; brain stimulation reward; rat; anandamide

INTRODUCTION

The brain eCB system is composed of lipid signaling molecules [2-arachidonoylglycerol (2-AG) and anandamide being the best characterized-, their G-protein coupled receptor targets (CB1 and CB2), enzymatic regulators and a recently identified transport system (Di Marzo, 2009; Fu et al., 2011). Disrupting eCB transmission using CB1 receptor antagonists is an effective approach to reduce aberrant reward seeking behavior. Indeed, clinical trials indicated that CB1 receptor antagonists effectively reduce food intake in obese patients (Scheen, 2008) and nicotine use in at-risk patients (Cahill and Ussher, 2007). Similar results are reported in the preclinical literature, particularly when the receipt of reward is dependent upon the guidance of conditioned cues (Vries and Schoffelmeer, 2005).

The nucleus accumbens (NAc) is a neural substrate involved in integrating environmental stimuli with emotional information to initiate reward-directed behavior (Mogenson et al., 1980). When presented with conditioned cues, subpopulations of NAc neurons exhibit distinct firing patterns, encoding information pertaining to the predicted reward. The therapeutic potential of drugs targeting the eCB system to reduce aberrant reward seeking is likely due, in part, to a disruption of NAc neural encoding of reward processing. We previously demonstrated that disrupting eCB signaling (2-AG exclusively, not anandamide) decreases NAc dopaminergic encoding of reward predictive cues (Oleson et al., 2012).

These findings led us to hypothesize that increasing eCB levels might strengthen NAc neural encoding of reward predictive cues and facilitate reward seeking. To assess the effects of

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increasing eCB levels on the neural mechanisms of reward seeking, we treated rats with cumulative doses of the eCB uptake inhibitor VDM11, while simultaneously recording extracellular single-unit activity in the NAc shell during operant behavior maintained by brain-stimulation reward in a cued-intracranial self-stimulation task.

METHODS AND RESULTS

Male Sprague-Dawley rats fitted with jugular vein catheters at vendor (Charles River) were used as subjects. All experiments were conducted during the light period. All procedures were performed in concordance with the University of Maryland, Baltimore's Institutional Animal Care and Use Committee (IACUC) protocols. Before the onset of experiments each rat was anesthetized with 2.0% isoflurane in O₂ and stereotactically implanted with a guide cannula above the NAc shell (+1.7mm AP, +0.8mm ML) a reference electrode in the contralateral hemisphere and a bipolar stimulating electrode in the ventral tegmental area. Upon recovery, a micromanipulator was attached to the guide cannula and used to lower a carbon fiber electrode into the NAc shell (Cheer et al., 2005). Following optimization of recording site, rats were given access to brain-stimulation reward under a fixed ratio 1 (FR1) schedule, as previously described (Oleson et al., 2012). Upon reaching criterion for acquisition (30 responses with a 10-s fixed timeout), the inter-trial interval was switched to occur variably (mean 30-s variable timeout). As in Oleson et al., 2012, under variable timeout conditions, trial onset was signaled to the animal by the extension of a lever and simultaneous presentation of a cue-light while in fixed timeout conditions the cue-light preceded lever extension by 1-sec. Each response resulted in retraction of the lever and dimming of the cue-light.

Variable timeouts were implemented to assess for drug-induced changes in NAc neural encoding of cue presentation because changes in patterned neural firing were strictly time-locked to cue presentation under these conditions. In contrast, as shown in Figure 1, patterned neural activity preceded cue presentation when the timing of reward availability was predictable. Thus, when assessing the effects of a drug on NAc neural encoding of a discrete environmental cue, it is critical to prevent the animal from timing reward availability.

After establishing stable behavior, the pharmacological component of the study commenced. Following 30 baseline responses, vehicle and then cumulative doses of VDM11 (300–560 µg/kg intravenous (IV) were administered; the dose-range and pretreatment time (0s) were determined in preliminary studies). Thirty responses were measured following each injection. VDM11 (Tocris, Ellisville, MO) was prepared daily in a 1:1:18 solution of ethanol, Emulphor and sterile saline. To control for repeated vehicle injections, we treated an additional group of rats with four vehicle injections in place of drug, an approach that failed to effect response latency or NAc encoding of cue presentation as previously reported (Oleson et al., 2012).

Neurons were identified as medium spiny neurons based on previously described criteria regarding waveform shape and firing rate (Nicola et al., 2004). Patterned neural activity was assessed by creating perievent rasters of firing rates surrounding cue-presentation and analyzed via bin-by-bin Z-score weighting. For statistics, data were first analyzed using the Shapiro-Wilk test for normality, then with either a one-way ANOVA or a Kruskal-Wallis one-way ANOVA on ranks depending on normality of distribution.

Under baseline conditions we observed significant NAc neural encoding of cue significance (defined as a z-score > 1 during the first second of cue presentation) in 5/6 rats. Two distinct

subpopulations of NAc neurons were detected; the more prominent (4/5) showed an inhibition time-locked to cue onset while a minority (1/5) showed an excitation.

VDM11 decreased reward seeking, defined as the time occurring between cue presentation and a reward-directed behavioral response, and NAc neural encoding of cue significance. Figure 2A illustrates the effects of VDM11 on the latency to respond for brain stimulation reward. A one-way ANOVA revealed a significant effect of VDM11 on response latency ($F_{(3,19)}=6.161$, $p<0.01$). Bonferroni post hoc analysis revealed that the 560 μ g/kg dose significantly increased response latency relative to vehicle ($t=3.706$, $p=0.012$). Figure 2B illustrates mean firing-rate histograms, aligned to cue presentation. Mean changes in neural encoding (expressed as absolute Z-scores) occurring during the first second of cue presentation (gray bar in Figure 2B) are illustrated in Figure 2C. A Kruskal-Wallis one-way ANOVA on ranks revealed a significant effect of VDM11 on Z-score ($H_{(4)}=34.63$, $p<0.01$). Tukey post hoc analysis revealed that the 420 ($q=4.664$, $p<0.05$) and 560 ($q=4.664$, $p<0.05$) μ g/kg dose significantly decreased Z-score in comparison to vehicle. All graphical representations of the data were created with Adobe Illustrator CS4 (Adobe Corp., San Jose, CA) and statistical analyses were performed with SigmaPlot v.11 (Systat Inc., San Jose, CA).

DISCUSSION

We were initially surprised to find that the eCB uptake inhibitor VDM11 reduced NAc neural encoding of reward-predictive cues similarly to our previous reports using CB1 receptor antagonists (Oleson et al., 2012). These counterintuitive findings may be explained by the alternative view that VDM11 increases anandamide to a greater degree than 2-AG *in vivo* (Van Der Stelt et al., 2006). VMD11-induced elevations of anandamide, which functions a partial CB1 receptor agonist, might compete with 2-AG, a full CB1 receptor agonist (Savinainen et al., 2001)—thereby preventing 2-AG from facilitating reward seeking. While both anandamide and 2-AG function as reinforcers in their own right when tested in squirrel monkeys (Justinova et al., 2005; Justinova et al., 2011), a growing body of evidence suggests that these molecules may exert opposing effects during particular circumstances of reward-directed behavior. Augmenting 2-AG levels increases dopamine neurotransmission (Oleson et al., 2012) and facilitates reward-directed behavior (Gutierrez-Lopez et al., 2010; Oleson et al., 2012; Wakley and Rasmussen, 2009). By contrast, augmenting anandamide levels reduces the potency of cues to motivate reward-directed behavior. For example, increasing anandamide levels by inhibiting its enzymatic degradation decreases both food and drug seeking behavior in reinstatement procedures (Adamczyk et al., 2009; Scherma et al., 2008) and drug-induced increases in NAc dopamine (Scherma et al., 2008). The similarities between the aforementioned results obtained using FAAH inhibitors and the data presented herein merits further discussion regarding the affinity of VDM11 for FAAH. Multiple *in vitro* studies report that VDM11 is also a substrate for FAAH, capable of reducing the metabolism of FAAH hydrolysis products (e.g., anandamide, palmitoylethanolamide and oleoylethanolamide) (Hillard et al., 2007; Vandevoorde and Fowler, 2005) but see (Bortolato et al., 2006; Fegley et al., 2004). Thus, it remains possible that the VDM11-induced dampening of the neural mechanisms of reward seeking might result not only from reduced anandamide uptake, but also from FAAH inhibition. In this case, it remains possible that other FAAH hydrolysis products, such as palmitoylethanolamide and oleoylethanolamide, might even potentiate the effects of anandamide at non-CB1 receptors, such as transient potential receptor of vanilloid type 1 (TRPV1) channels (van der Stelt et al., 2005). Indeed, a previously proposed theory posits that anandamide activation of TRPV1 receptors may oppose activation of CB1 receptors (Di Marzo, 2010). As the specific pharmacological mechanisms of action at play remain unclear,

local administration of selective endocannabinoid uptake inhibitors may reveal findings that are distinct from those reported herein (Soria-Gomez et al., 2007).

Yet a further interpretation regarding the pharmacological mechanism of action by which VDM11 decreased the neural mechanisms of reward seeking involves the potential bidirectional nature of a promiscuous endocannabinoid transporter (Bisogno et al., 2001; Di et al., 2005; Hajos et al., 2004). Specifically, if the endocannabinoid transport system is involved in the bidirectional transport of both 2-AG and anandamide, as has been suggested (Ligresti et al., 2004; Melis et al., 2004; Ronesi et al., 2004; van der Stelt et al., 2005), pharmacological compounds designed to inhibit the uptake of endocannabinoids may actually produce effects that are reminiscent to those observed following CB1 receptor blockade as was found in the present study.

Our current data confirm recent behavioral findings, showing that eCB uptake inhibitors decrease reward seeking (Gamaleddin et al., 2011; Scherma et al., 2011; Vlachou et al., 2006) similarly to other drugs that selectively augment anandamide levels or antagonize CB1 receptors. Our electrophysiological data showing that the post-synaptic NAc neural response to conditioned cues is diminished by VDM11 offers a previously undocumented neural mechanism explaining how eCB uptake inhibitors effectively weaken reward seeking. These findings demonstrate that eCB uptake inhibitors dampen the neural mechanisms of cue-motivated behavior and offer new insights into the search for an endocannabinoid drug target that might treat disorders of motivation while producing limited side effects.

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Fig. 1. Patterned NAc neural activity precedes cue presentation when reward availability is predictable (Fixed timeout; top) but is time-locked to cue-presentation when reward availability occurs randomly (Variable timeout; bottom).

Fig. 2.

Using a cumulative dosing-approach, VDM11 dose-dependently decreased reward seeking and NAc neural encoding of reward significance. 30 responses occurred following each drug-infusion. **A:** VDM11 increased the latency occurring between conditioned cue presentation and a reward-directed lever response. **B:** VDM11 decreased patterned neural activity beginning at cue onset (gray bar: first second after cue). **C:** VDM11 dose-dependently decreased mean NAc neural encoding during the first second of cue presentation. Error bars represent SEM. Asterisk (*) indicates significance relative to vehicle at $p < 0.05$.