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Short communication

GABAergic blockade of cocaine-associated cue-induced increases in nucleus accumbens dopamine

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Abstract

Environments previously associated with drug use can become one of the most common factors triggering relapse to drug-seeking behavior. To better understand the neurochemical mechanisms potentially mediating these cues, we measured nucleus accumbens dopamine levels in animals exposed to environmental cues previously paired with cocaine administration. In animals exposed to a cocaine-paired environment nucleus accumbens dopamine increased by 25%. When administered 2.5 h prior to presentation of the environmental trigger, racemic vigabatrin (an irreversible inhibitor of γ-aminobutyric acid (GABA)-transaminase) abolished this cue-induced increase. Conversely, *R*-(−)-vigabatrin, the inactive enantiomer, had no effect. Combined with our earlier findings, these studies support the potential therapeutic benefit of this enzyme-based GABAergic strategy to modulate brain dopamine and the subsequent treatment of drug addiction.

Keywords: Cocaine; Conditioning; Contextual cue; Microdialysis, in vivo; GABA (γ-aminobutyric acid); Vigabatrin

1. Introduction

In view of the well-established role of the mesocorticolimbic system in reinforcement and reward (Leshner and Koob, 1999), the action of addictive drugs on nucleus accumbens dopamine has been regarded as a key neurobiological substrate of addiction. It is also well documented that exposure to salient environmental cues formerly associated with drug-taking behavior can produce intense craving (Grant et al., 1996) and become powerful triggers to relapse in cocaine abusers (Childress et al., 1988). Contextual cues associated with drug intake also trigger relapse in animals behaviorally extinguished from previous drug-taking in "reinstatement" paradigms, which serves as an animal model of relapse (Meil and See, 1996). Importantly, cue-triggered relapse appears critically dependent on brain dopamine mechanisms. For example, pharmacological blockade of dopamine D_1 and D_2 receptors attenuates cue-triggered relapse to cocaine-seeking behavior (Maldonado-Irizarry et al., 1996).

However, the degree to which dopamine mediates specific behaviors triggered by these environmental cues is unclear. For example, Brown and Fibiger (1992) used in vivo brain microdialysis to demonstrate found cocaine-paired environmental cues elicited conditioned locomotion in rats, without an increase in nucleus accumbens dopamine. Bradberry et al. (2000) found no increases in ventromedial striatal dopamine in rhesus monkeys exposed to a visual cue that had previously indicated availability of intravenous cocaine. On the other hand, Di Chiano et al. (1998) found that a conditioned stimulus previously paired with administration of intravenous cocaine produced significant augmentation of dopamine oxidation currents in

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nucleus accumbens. Similarly, Weiss et al. (2000) used the "reinstatement" paradigm to show that exposure to a drug-associated discriminative cue significantly increased dopamine efflux in both the nucleus accumbens and amygdala.

We have studied γ-aminobutyric acid (GABA)/dopamine interactions using the irreversible inhibitor of GABA transaminase, the primary enzyme involved in catabolism of GABA. Gamma-vinyl-GABA (GVG, vigabatrin), by interfering with this process, significantly enhances brain GABA levels. We have reported that GVG dose-dependently attenuates increases in nucleus accumbens dopamine produced by several drugs of abuse, including cocaine (Dewey et al., 1998; Gerasimov et al., 1999) as well as decreases cocaine self-administration behavior in rats (Kushner et al., 1999).

While many investigations of this kind use discrete conditioned stimuli, typically a light temporally associated with drug self-administration, we have chosen to use the contextual cues signaling general availability of a drug utilized in the conditioned place preference that have proven to be highly robust at eliciting drug-conditioned approach behavior (Schechter and Calcagnetti, 1998). Therefore, the present investigation was undertaken to study the effect on nucleus accumbens dopamine of conditioned place preference-like drug-associated contextual cues and to investigate modulatory effects of enhanced GABAergic tone on cue-evoked changes in nucleus accumbens dopamine.

2. Materials and methods

Male Sprague–Dawley rats were used in all experiments (200–300 g, Taconic Farms, Germantown, NY) and were housed individually on a 12/12 h light/dark cycle. Animals were anesthetized with a ketamine/xylazine mixture and siliconized guide cannulae were stereotaxically implanted into the right nucleus accumbens (1.5 mm anterior and 1.0 mm lateral to bregma, and 5.6 mm ventral to the cortical surface; Paxinos and Watson, 2nd edn., 1986). Animals recovered from surgery for 3 days prior to handling and conditioning procedures.

Cocaine hydrochloride (20 mg/kg, Sigma, St. Louis, MO), racemic (+/−) and R-(−) vigabatrin (300 mg/kg, 4-amino-hex-5-enoic acid, ChiroTech Technology, Cambridge, UK) were dissolved in saline and administered by intraperitoneal (i.p.) injection 2.5 h prior to presentation of the cocaine-paired environment.

The conditioned place preference paradigm used in our previous studies (Dewey et al., 1998) was modified in the following manner. The conditioning environment was a Plexiglas bowl with black-and-white stripes and a bare floor or white walls with corncob bedding. Following two habituation sessions, all animals underwent 30 min training sessions that included receiving either cocaine or saline immediately prior to being placed into either paired environment. Animals who received cocaine in the white bowl on the first day received saline in the striped bowl on the following day and visa versa. Thus, all animals received four cocaine and four saline injections over 8 consecutive days. The control group received saline injections in both bowls over the same 8-day period. On the test day, microdialysis probes were inserted into the guide cannulae, animals were placed in the non-paired environment and baseline measurements of nucleus accumbens dopamine were obtained as defined by at least four consecutive dopamine samples differing by not more than 10%. Upon establishing this baseline, Groups 1 and 2 animals received saline, Group 3 animals received racemic vigabatrin, and Group 4 animals received *R*-(−)-vigabatrin followed 2.5 h later by presentation of the individually appropriate cocaine-paired environment. Dialysis samples were injected on-line every 5 min for 40 min while the animal remained in the bowl.

Thus, treatment schedule was as follows:

Group 1 (control) received only saline injections on the training days and on the test day.
Groups 2, 3 and 4 all underwent cocaine/saline treatment on the training days, with Group 2 receiving saline, Group 3—racemic vigabatrin and Group 4—*R*-(−) vigabatrin on the test day.

Microdialysis probes (2.0 mm, Bioanalytical Systems, BAS, West Lafayette, IN) were perfused with Ringer's solution (McGaw, Inc., Irvine, CA) at a flow rate of 2.0 μl/min. The high-pressure liquid chromatography (HPLC) system consists of a BAS reverse-phase column (3.0 μC-

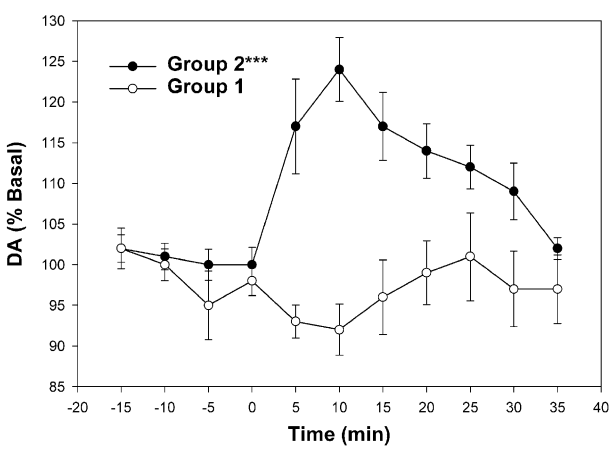

Fig. 1. *Increases in extracellular nucleus accumbens dopamine levels produced by exposure to cues previously paired with cocaine administration in freely moving animals*. Values are expressed as percentage of baseline dopamine and represent the mean ± S.E. ($n = 6–9$/group). Exposure to conditioned environment started at time 0. *** Values are significantly different from animals in Group 1 (control group treated with saline during conditioning trials, $P < 0.0001$, ANOVA and Student's *t*-test).

18) and a BAS LC-4C electrochemical transducer with a dual glassy carbon electrode set at +650 mV. The mobile phase consisted of 11% acetonitrile, 50 mmol sodium phosphate monobasic, 1.0 mmol sodium octyl sulfate, and 0.1 mmol EDTA, pH 4.0. Dopamine eluted at 4 min.

Statistical and graphical analyses of data from studies comparing the magnitude of the dopaminergic response were expressed as percentages of baseline levels. To compare the effects of pretreatment, a two-tailed Student's *t*-test was performed on all data intervals within the 60-min sampling period assuming equal variance. A single-factor analysis of variance (ANOVA) provided significance for differences across all treatment groups, where necessary. Post-hoc analysis was performed using Scheffe's test, and critical values for the *F*-statistic were evaluated at alpha = 0.05.

3. Results

In control studies (Group 1, $n = 9$), saline injections administered 2.5 h prior to presentation of the experimental environmental did not produce significant changes in extracellular nucleus accumbens dopamine. However, in Group 2 animals, exposure to the cocaine-paired environment was accompanied by an increase in dopamine levels, which reached a maximal value of 24% above baseline within 10 min and were significantly greater than the values for the control group (Group 1) ($t = -4.89$, $P < 0.0001$) (Fig. 1).

Vigabatrin (300 mg/kg) administered 2.5 h prior to presentation of the environmental trigger (Group 3) completely abolished cue-induced increases in nucleus accumbens dopamine (Fig. 2). That is, extracellular dopamine levels in vigabatrin pretreated animals were not significantly different from the control (saline) group values (Group 1; $t = 0.062$, $P = 0.5137$), were significantly different from cocaine-conditioned animals who received saline on the test day (Group 2) ($t = 7.29$, $P < 0.0001$). However, when *R*-(−)-vigabatrin was administered 2.5 h prior to presentation of the cocaine-paired environment (Group 4), increases in nucleus accumbens dopamine were not statistically different from those obtained in animals receiving saline on the test day (Group 2) ($t = 0.3$, $P = 0.76$), but were significantly different from those animals treated with racemic vigabatrin ($t = 5.54$, $P < 0.0001$). ANOVA followed by Scheffe's test revealed significant differences between those animals pretreated with GVG and those pretreated with saline or *R*-(−)-vigabatrin ($F = 19.54$, $P < 0.0001$).

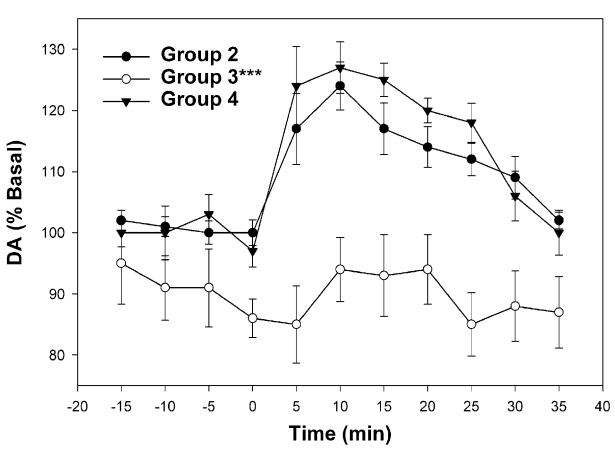

Fig. 2. *Effect of pretreatment with racemic (Group 3) or R-(−) vigabatrin (Group 4) (300 mg/kg) on cue-induced increases in nucleus accumbens dopamine.* Values are expressed as percentage of baseline dopamine and represent the mean ± S.E. ($n = 6–8$/group). Exposure to conditioned environment started at time 0. *** Values obtained from cocaine-conditioned animals treated with racemic vigabatrin (Group 3) are significantly different from those treated with saline on the test day (Group 2) and from those treated with *R*-(−) vigabatrin (Group 4, $P < 0.0001$, ANOVA and Student's *t*-test).

4. Discussion

In the present study we observed increases in nucleus accumbens dopamine in response to cocaine-paired contextual cues. The current findings are consistent with an important role for nucleus accumbens dopamine in mediating incentive motivation and approach behavior (Berridge and Robinson, 1998), and are in agreement with the theoretical formulation of Stewart et al. (1984) that environmental stimuli associated with the administration of addictive drugs can come to elicit neural states similar to those produced by the drugs themselves. We also suggest that our findings support proponent-process (Stewart and Wise, 1992) as opposed to opponent-process theories of drug craving and relapse. Proponent-process theories postulate that drug-seeking behavior is primed by drug-like actions of environmental cues and other stimuli that provoke relapse; opponent-process theories postulate that drug-seeking behavior is primed by drug-opposite effects associated primarily with drug withdrawal.

That drug-associated environmental cues evoked significant enhancement of nucleus accumbens dopamine may appear contradictory to the recent demonstration (Grimm and See, 2000) that the nucleus accumbens is critical to *drug*-triggered reinstatement of cocaine-seeking behavior while the basolateral amygdala is involved in *cue*-triggered reinstatement. We would note, however, that current understandings of reward learning require that the neural substrates of unconditioned and conditioned reinforcers must converge in some final common neural pathway. We would further point out that Grimm and See used a discrete conditioned stimulus, while we used CPP-like environmental contextual cues. Additionally, our findings are conceptually consistent with the report (Franklin and Druhan, 2000) that a cocaine-associated environment produced significant augmentation of Fos-related antigen expression in both the nucleus accumbens shell and core.

Overall, cue-evoked activation of nucleus accumbens substrates appears consistent with other evidence that the nucleus accumbens plays an important role in conditioned responding to a variety of stimuli associated with drug reinforcement (Everitt et al., 1991).

Administration of racemic vigabatrin completely abolished the cue-evoked increases in nucleus accumbens dopamine. We suggest that augmentation of GABAergic inputs to the ventral tegmental area-nucleus accumbens axis blunts dopamine tone within that system, but does not fully inhibit dopamine function. For example, vigabatrin does not produce catalepsy in rodents nor does it significantly alter locomotion. In this regard, we would note that vigabatrin does not produce inhibition of a conditioned place preference for food reward while it inhibits both the acquisition and expression of cocaine-induced conditioned place preference (Dewey et al., 1998). Thus, vigabatrin blunts the drug-seeking effects produced by the stronger dopamine-enhancing stimulus (cocaine) while leaving unaffected the appetitive effects of a weaker dopamine-enhancing stimulus (food).

While the present experiment was strictly biochemical in terms of its dependent variable, we believe that our findings are related to drug-seeking behavior. First, as noted above, Weiss et al. (2000) have found that cocaine-associated cue-evoked augmentation of nucleus accumbens dopamine is correlated with relapse to cocaine-seeking behavior. Second, intra-nucleus accumbens microinjections of addictive drugs augment drug-seeking behavior (Stewart and Vezina, 1988). Finally, nucleus accumbens dopamine appears strongly involved in mediating aspects of conditioned place preference drug-seeking behavior (McBride et al., 1999).

It is in this context that we believe the present demonstration of pharmacological blockade of environmental cue-induced increases in extracellular nucleus accumbens dopamine has potential clinical implications. Biochemical and behavioral responses to environmental cues frequently mimic those associated with the drug itself and act as primers to subsequent drug self-administration. These phenomena are often invoked to explain relapse in humans (Robbins and Ehrman, 1992). At present, there are no medications known to be clinically effective against cue-induced craving or relapse. Recently, the novel partial dopamine D_3 receptor agonist has been shown to inhibit cocaine-seeking behavior in the presence of drug-paired cues (Pilla et al., 1999). However, this drug appears unable to reduce the reinforcing effects of cocaine, whereas vigabatrin is.

The present finding that racemic, but not *R*-(−)-vigabatrin, blocked cue-induced increases in nucleus accumbens dopamine is consistent with our earlier work demonstrating that racemic and *S*-(+)-vigabatrin, but not *R*-(−)-vigabatrin, effectively inhibited nicotine- and cocaine-induced increases in mesolimbic dopamine (Schiffer et al., 2000). We therefore believe that this stereoselectivity supports the need for further evaluation of the single enantiomer and continued exploration of the enzyme-based GABAergic approach for the modulation of brain dopamine and subsequent treatment of drug addiction.

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