Synergistic Self-Administration of Ethanol and Cocaine Directly into the Posterior Ventral Tegmental Area: Involvement of Serotonin-3 Receptors

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ABSTRACT

Ethanol (EtOH) and cocaine are both self-administered into the posterior ventral tegmental area (VTA). Self-administration of either drug is prevented by coadministration of a serotonin (5-HT₃) receptor antagonist. Electrophysiological studies indicated that cocaine and EtOH can act synergistically to stimulate VTA dopamine neurons. The current experiment assessed whether cocaine and EtOH would synergistically interact to produce a reinforcing action within the posterior VTA. Adult female Wistar rats were randomly assigned to one of 13 groups. There were three control groups: artificial cerebrospinal fluid (aCSF), a subthreshold EtOH (100 mg%) group, and a subthreshold cocaine (25 pmol/100 nl) group. The other groups self-administered 50 or 75 mg% EtOH containing 6.25, 12.5, or 25 pmol/100 nl cocaine or 100 mg% EtOH containing 3.12, 6.25, 12.5, or 25 pmol/100 nl cocaine. All rats received the

assigned infusate for sessions 1 through 4, aCSF alone in sessions 5 and 6, and the original infusate during session 7. The effects of adding a 5-HT₃ receptor antagonist [tropisetron, C₁₇H₂₀N₂O₂ (ICS 205,930) and C₁₇H₂₂N₄O.C₄H₄O₄ (LY278-584)] on coadministration of EtOH and cocaine (75 mg% + 12.5 pmol/100 nl) were determined. Rats failed to self-administer aCSF or the subthreshold concentration of EtOH or cocaine. All three concentrations of EtOH (50, 75, and 100 mg%) combined with cocaine (12.5 and 25 pmol/100 nl) supported self-administration. Adding a 5HT₃ receptor antagonist attenuated coadministration of EtOH + cocaine. Overall, the data indicate that the reinforcing properties of EtOH and cocaine interacted synergistically within the posterior VTA, and these synergistic effects were mediated, at least in part, by activation of local 5-HT₃ receptors.

Introduction

The coabuse of alcohol and cocaine has been frequently reported in humans. A majority of cocaine dependents can be diagnosed as alcohol dependents (Miller et al., 1989; Carroll et al., 1993). Simultaneous use of cocaine and alcohol elicited greater euphoria-like subjective effects than use of either drug alone (Farré et al., 1997). Alcoholics were more likely to use cocaine, and cocaine use increased alcohol consumption (Heil et al., 2001; Staines et al., 2001). Furthermore, there is a genetic linkage between alcohol and cocaine dependence in humans, as indicated by higher rates of cocaine abuse and

dependence in first-degree relatives of alcoholics (Nurnberger et al., 2004).

Animal research also indicates an interaction between alcohol and cocaine. Alcohol-preferring rats seemed to be more sensitive than Wistar rats to the reinforcing effects of cocaine in the nucleus accumbens (NAC) shell (Katner et al., 2011) and be more sensitive than alcohol-nonpreferring rats to drug-induced reinstatement of cocaine-seeking behavior (Lê et al., 2006). High alcohol-consuming Wistar rats were more sensitive to the rewarding effects of cocaine than low alcohol-consuming Wistar rats (Stromberg and Mackler, 2005). Repeated administration of cocaine induced sensitization to the dopamine-releasing effects of cocaine in alcohol-preferring alko alcohol rats but not in alcohol-avoiding alko non-alcohol rats (Mikkola et al., 2001). Furthermore, long-term EtOH exposure produced an enhanced locomotor sensitization to cocaine (Manley and Little, 1997). Repeated administration of cocaine produced cross-sensitization of mice to the locomo-

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ABBREVIATIONS: NAC, nucleus accumbens; aCSF, artificial cerebrospinal fluid; VTA, ventral tegmental area; ICS 205,930, tropisetron, C₁₇H₂₀N₂O₂; LY278-584, C₁₇H₂₂N₄O.C₄H₄O₄; ANOVA, analysis of variance.

tor stimulation effects of EtOH and vice versa (Itzhak and Martin, 1999). Acquisition of intravenous cocaine self-administration was positively correlated with levels of operant self-administration of EtOH in rats (Mierzejewski et al., 2003).

By use of the intracranial self-administration technique, studies have shown that cocaine and EtOH were self-administered by rats into the posterior but not anterior VTA (Rodd et al., 2004, 2005) and the NAC shell but not core (Rodd-Henricks et al., 2002; Engleman et al., 2009), suggesting that the posterior VTA and NAC shell are two common brain regions supporting the reinforcing effects of both EtOH and cocaine. Functional magnetic resonance-imaging studies indicated that short-term exposure to cocaine or EtOH-related olfactory cues activated the VTA in humans (Breiter et al., 1997; Kareken et al., 2004). The reinforcing effects of EtOH or cocaine in the posterior VTA require activation of local dopamine neurons in rats (Rodd et al., 2004, 2005). EtOH can stimulate mesocorticolimbic dopamine neurons in the posterior VTA (Brodie et al., 1995) and increase dopamine release in terminal regions (Ding et al., 2009a, 2011). Short-term administration of cocaine or EtOH produced similar synaptic plasticity on VTA dopamine neurons involving glutamate synapses (Saal et al., 2003). Electrophysiological studies also indicated that low doses of cocaine can potentiate EtOH-induced excitation of dopamine neurons in VTA slices (Bunney et al., 2000, 2001). However, no animal study has examined the coadministration of cocaine and EtOH in rats. Given the positive interaction between EtOH and cocaine, the current study tested the hypothesis that EtOH and cocaine could produce synergistic reinforcing effects within the posterior VTA.

5-HT₃ receptors within the posterior VTA have been implicated in the effects of EtOH and cocaine. Local application of a 5-HT₃ receptor antagonist attenuated the EtOH-induced increase of extracellular dopamine levels within the mesocorticolimbic system (Ding et al., 2011). In addition, repeated injections of a 5-HT₃ receptor antagonist in the posterior VTA altered the acquisition and maintenance of operant self-administration of EtOH in alcohol-preferring rats (Rodd et al., 2010). Furthermore, coinjection of 5-HT₃ receptor antagonists attenuated the self-infusion of both EtOH and cocaine into the posterior VTA (Rodd-Henricks et al., 2003; Rodd et al., 2005). Given these findings, the second objective of the current study was to examine the involvement of local 5-HT₃ receptors in the coadministration of EtOH and cocaine in the posterior VTA. The hypothesis to be tested was that the synergistic effects of EtOH and cocaine within the posterior VTA are mediated, at least in part, by activation of 5-HT₃ receptors.

Materials and Methods

Animals. Adult female Wistar rats (Harlan, Indianapolis, IN) weighing 250 to 320 g at the time of surgery were used. Animals were pair-housed upon arrival and maintained on a 12-h reverse light/dark cycle (lights off at 9:00 AM). The estrous cycle was not monitored in the present study. However, counterbalanced experiments were conducted on different days so that any effect of a given phase of the estrous cycle was distributed across experimental conditions. In addition, the current and previous studies (Rodd et al., 2004; Ding et al., 2009b) did not observe obvious fluctuations in ICSA behavior in rats that were given similar doses of the same agent for two or more sessions conducted every other day. The main benefit of using

female rats is that they maintain their head size better for more accurate stereotaxic placements. For EtOH self-administration in the posterior VTA, previous results indicated that male and female Wistar rats were similar (Rodd-Henricks et al., 2003; Rodd et al., 2004). Food and water were freely available, with the exception of the test chamber. All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

The number of animals indicated for each experiment represents approximately 95% of the total number that underwent surgery; 5% of the animals was not included for analyses, mainly because of the loss of the guide cannula before completion of all experimental sessions. The data for these animals were not used because their injection sites could not be verified.

Drugs. The artificial cerebrospinal fluid (aCSF) vehicle consisted of 120.0 mM NaCl, 4.8 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 2.5 mM CaCl₂, and 10.0 mM *D*-glucose. EtOH (C₂H₆O; McCormick Distilling Co., Inc., Weston, MO), cocaine HCl (C₁₇H₂₁NO₄; National Institute on Drug Abuse), ICS 205,930 (tropisetron, C₁₇H₂₆N₂O₂; Sigma-Aldrich, St. Louis, MO), and LY278-584 maleate (C₁₇H₂₂N₄O₄; Sigma-Aldrich) were dissolved in the aCSF solution to the desired concentrations. When necessary, 0.1 M HCl or 0.1 M NaOH was added to the solutions to adjust pH levels to 7.4 ± 0.1.

Apparatus. The test chambers (30 × 30 × 26 cm; width × height × diameter) were situated in sound-attenuating cubicles (64 × 60 × 50 cm; Coulbourn Instruments, Allentown, PA), which were illuminated by a dim house-light during testing. Two identical levers (3.5 × 1.8 cm) were mounted on a single wall of the test chamber, 15 cm above a grid floor, and were separated by 12 cm. Levers were raised to this level to avoid accidental brushing against the lever and to reduce responses as a result of locomotor activation. There was a row of three different colored cue lights directly above each lever. The light (red) to the far right over the active bar was illuminated during resting conditions. A desktop computer equipped with an operant control system (L2T2 system; Coulbourn Instruments) recorded the data and controlled the delivery of the infusate in relation to lever response.

An electrolytic microinfusion transducer system (Ding et al., 2009b) was used to control microinfusions of drug or vehicle. In brief, two platinum electrodes were placed in an infusate-filled cylinder container (28 mm in length × 6 mm in diameter) equipped with a 28-gauge injection cannula (Plastics One, Roanoke, VA). The electrodes were connected by a spring-coated cable (Plastics One) and swivel (model 205; Mercotac, Inc., Carlsbad, CA) to a constant current generator (MNC Inc., Shreveport, LA) that delivered 6 μA of quiescent current or 200 μA of infusion current between the electrodes. Depression of the active lever delivered the infusion current for 5 s, which led to the rapid generation of H₂ gas (raising the pressure inside the airtight cylinder) and, in turn, forced 100 nl of the infusate through the injection cannula. During the 5-s infusion and additional 5-s timeout period, the house light and right cue light (red) were extinguished, and the left cue light (green) over the active lever flashed on and off at 0.5-s intervals.

Stereotaxic Surgery. While under isoflurane anesthesia, a unilateral 22-gauge guide cannula (Plastics One) was stereotaxically implanted in the right hemisphere of each subject, aimed 1.0 mm above the posterior VTA (anteroposterior +5.6 mm, mediolateral +2.1 mm, dorsoventral −8.5 mm) at a 10° angle to the vertical. In between experimental sessions, a 28-gauge stylet was placed into the guide cannula and extended 0.5 mm beyond the tip of the guide. After surgery, all rats were individually housed and allowed to recover 7 to 10 days. Animals were handled for at least 5 min daily after the 4th recovery day. Subjects were not acclimated to the test

chamber before the commencement of data collection, nor were they trained on any other operant paradigm.

General Test Condition. For testing, subjects were brought to the testing room, the stylet was removed, and the injection cannula screwed into place. Rats were placed individually in the test chamber. To avoid trapping air at the tip of the injection cannula, the infusion current was delivered for 5 s during insertion of the injector that resulted in a noncontingent administration of the drug or aCSF at the beginning of the session. Injection cannulae extended 1.0 mm beyond the tip of the guide. The test chamber was equipped with two levers. Depression of the “active lever” (fixed ratio 1 schedule of reinforcement) caused the delivery of a 100- μ l bolus of infusate over a 5-s period followed by a 5-s time-out period. During both the 5-s infusion period and 5-s time-out period, responses on the active lever did not produce further infusions. The assignment of active and inactive lever with respect to the left or right position was counter-balanced among subjects. However, the active and inactive levers remained the same for each rat throughout the experiment. No shaping technique was used to facilitate the acquisition of lever responses. The number of infusions and responses on the active lever was recorded. Responses on the “inactive lever” were recorded but did not result in infusions. The duration of each test session was 4 h, and sessions occurred every other day.

EtOH-Cocaine Coadministration. Animals were randomly assigned to one of 13 groups ($n = 8$ –10/group). A vehicle group received infusions of aCSF alone for all seven sessions. Separate groups of rats received a subthreshold concentration of EtOH (100 mg%; Rodd-Henricks et al., 2000) or cocaine alone (25 pmol/100 μ l; Rodd et al., 2005). The other 10 groups were allowed to self-administer the mixture of EtOH and cocaine. Rats were allowed to self-administer 50 or 75 mg% EtOH containing 6.25, 12.5, or 25 pmol/100 μ l cocaine. Additional groups of rats were allowed to self-administer 100 mg% EtOH containing 3.12, 6.25, 12.5, or 25 pmol/100 μ l cocaine. The original infusate solution was available for self-administration during the first four sessions. During the fifth and sixth sessions, all animals received infusions of aCSF alone. On the seventh session, rats were allowed to respond for their originally assigned infusate.

Coinfusion of 5-HT₃ Antagonists with 75 mg% EtOH + 12.5 pmol/100 μ l Cocaine. Previous research indicated that coadministration of a 5-HT₃ receptor antagonist could attenuate self-administration of EtOH or cocaine alone into the posterior VTA (Rodd-Henricks et al., 2003; Rodd et al., 2005). Administration of 5-HT₃ receptor antagonists into the posterior VTA did not result in a reduction of locomotor activity, was self-administered at a comparable level of aCSF, and did not alter oral operant self-administration of saccharin (Rodd-Henricks et al., 2003; Rodd et al., 2010).

The 5-HT₃ receptor antagonists tested in the current study were ICS 205,930 and LY278-584. Rats were randomly assigned to one of six groups ($n = 5$ –6/group). All groups self-administered the mixture of 75 mg% EtOH plus 12.5 pmol/100 μ l cocaine for the initial four sessions, 75 mg% EtOH and 12.5 pmol/100 μ l cocaine with one concentration of either ICS 205,930 (50, 100, or 200 μ M) or LY278-584 (25, 50, or 100 μ M) during sessions 5 and 6, and the mixture of 75 mg% EtOH plus 12.5 pmol/100 μ l cocaine alone during session 7.

Histology. At the end of each experiment, 1% bromphenol blue (0.5 μ l) was injected into the infusion site. Subsequently, the animals were euthanized with an overdose of CO₂ inhalation and then decapitated. Brains were removed and immediately frozen at -70°C . Frozen brains were equilibrated at -15°C in a cryostat microtome and then sliced into 40- μ m sections. Sections were then stained with cresyl violet and examined under a light microscope for verification of the injector site using the rat brain atlas of Paxinos and Watson (1998).

Statistical Analysis. Data are expressed as mean $\pm$ S.E.M. For infusion data, numbers of infusions were averaged during the first four acquisition sessions. One-way ANOVAs were conducted with “group” as the between-subject factor followed by Tukey’s B post hoc analysis when a significant main effect was observed.

For lever response data, an overall mixed ANOVA with group and “lever” (active versus inactive) as the between-subject factors and “session” (1–7) as the within-subject factor was conducted. To decompose the significant interaction term (group $\times$ lever $\times$ session), the group factor was held constant, and individual mixed ANOVAs (lever $\times$ session) were conducted for each individual group to determine lever discrimination followed by “paired t ” tests in each individual group comparing responses between active and inactive lever during individual sessions. Lever discrimination is a key factor when a drug is self-administered (e.g., EtOH, cocaine, and amphetamine) to distinguish between reinforcement-contingent behavior and drug-stimulated locomotor activity.

Results

The posterior VTA was defined as the VTA region at coronal sections from -5.3 to -6.04 mm to bregma (Ding et al., 2009a; Fig. 1). Cannula placements surrounding the VTA

Fig. 1. Illustration depicts the injection sites indicated as solid triangles in the posterior VTA (defined as -5.3 to -6.0 mm bregma) of female Wistar rats self-administering aCSF alone, 100 mg% EtOH alone, 25 pmol/100 μ l cocaine alone, or various mixtures of cocaine and EtOH. For clarity purpose, overlapping injection sites are not included in the illustration. Injection sites indicated as solid circles outside the posterior VTA are mainly in the substantia nigra and red nucleus. These rats were not included in the analysis.

included injection sites located in the substantia nigra and red nucleus. Rats with injector tip placements outside the posterior VTA ($n = 5$) displayed an overall low level of infusions and active lever responding throughout all sessions (average infusions and active lever responses for the initial four sessions were 8 ± 1 and 16 ± 1 , respectively). For all sessions, the number of infusions of the combination of EtOH and cocaine outside the posterior VTA was not significantly different from the aCSF group with injection sites in the posterior VTA ($P > 0.53$). Likewise, examination of the active lever responses revealed that rats administering the mixtures of EtOH and cocaine into areas outside the posterior VTA displayed equivalent amounts of low levels of responding on both the active and inactive levers ($P > 0.55$).

EtOH-Cocaine Coadministration. The subthreshold doses of cocaine (25 pmol/100 nl) and EtOH (100 mg%) were self-administered at a comparable rate as aCSF (Fig. 2A). The mixture of EtOH and cocaine supported the development of self-administration behaviors. An ANOVA on the average number of infusions (Fig. 2) during the initial four acquisition sessions revealed a significant effect of group ($F_{12,101} =$

25.6; $P < 0.0001$). Tukey's B post hoc comparisons indicated that rats self-infusing the mixture of 50 mg% EtOH + 12.5 or 25 pmol/100 nl cocaine (Fig. 2B); 75 mg% EtOH + 12.5 or 25 pmol/100 nl cocaine (Fig. 2C); or 100 mg% EtOH + 6.25, 12.5, or 25 pmol/100 nl cocaine (Fig. 2D) received more infusions than rats given aCSF, 25 pmol/100 nl cocaine alone and 100 mg% EtOH alone or the mixture of 50 or 75 mg% EtOH + 6.25 pmol/100 nl cocaine or 100 mg% EtOH + 3.12 pmol/100 nl cocaine (Fig. 2). In addition, rats self-infusing the mixture of 100 mg% EtOH + 12.5 pmol/100 nl cocaine received more infusion than all other 100 mg% EtOH and cocaine combination groups ($P < 0.05$; Fig. 2D).

The repeated measure ANOVA performed on lever responses (active and inactive) for all 13 groups indicated a significant session $\times$ lever $\times$ group interaction ($F_{72,558} = 2.36$; $P < 0.0001$; Figs. 3–5). In general, rats given aCSF (Fig. 3, top), 100 mg% EtOH (Fig. 3, bottom) and 25 pmol/100 nl cocaine (Fig. 3, middle) or the mixture of 50 or 75 mg% EtOH + 6.25 pmol/100 nl cocaine (data not shown) or 100 mg% EtOH + 3.12 pmol/100 nl cocaine (Fig. 5, top left) responded on the active and inactive levers at comparable low levels during all sessions. There were no significant effects of session, lever, or session $\times$ lever interaction (all P values > 0.05) in these groups.

During sessions 1 through 4, rats given the mixture of 50 mg% EtOH + 12.5 or 25 pmol/100 nl cocaine (Fig. 4, left), 75

Fig. 2. The average number of infusions ($\pm$ S.E.M.) across the initial four sessions (acquisition) as a function of infusate concentration ($n = 8$ –10/group). A, average infusions of aCSF alone, 100 mg% EtOH alone, and 25 pmol/100 nl alone. B and C, average infusions of 50 or 75 mg% EtOH containing 6.25, 12.5, or 25 pmol/100 nl cocaine. D, average infusions of 100 mg% EtOH containing 3.12, 6.25, 12.5, or 25 pmol/100 nl cocaine. *, $P < 0.05$, significantly higher than aCSF, 25 pmol/100 nl, 100 mg% EtOH, and the mixtures of EtOH and the lowest concentration of cocaine; #, $P < 0.05$, significantly higher than the other 100 mg% EtOH and cocaine combination groups.

Fig. 3. The number of active and inactive lever responses (means $\pm$ S.E.M.) for rats self-administering aCSF alone (top), 25 pmol/100 nl cocaine alone (middle), or 100 mg% EtOH alone (bottom) into the posterior VTA during sessions 1 through 4, aCSF for sessions 5 and 6, and original infusate during session 7.

Fig. 4. The number of active and inactive lever responses (means $\pm$ S.E.M.) for rats self-administering a mixture of 50 (left) or 75 mg% (right) EtOH and cocaine (12.5–25 pmol/100 nl) into the posterior VTA during sessions 1 through 4, aCSF alone for sessions 5 and 6, and the original infusate mixture during session 7. *, $P < 0.05$, significant difference from responses on the inactive lever.

Fig. 5. The number of active and inactive lever presses (means $\pm$ S.E.M.; $n = 8-10$ /group) for rats self-administering a mixture of EtOH (100 mg%) and cocaine (3.1–25 pmol/100 nl) into the posterior VTA during sessions 1 through 4, aCSF alone for sessions 5 and 6, and original infusate mixture during session 7. *, $P < 0.05$, significantly higher than responses on the inactive lever.

mg% EtOH + 12.5 or 25 pmol/100 nl cocaine (Fig. 4, right), or 100 mg% EtOH + 6.25, 12.5, or 25 pmol/100 nl cocaine (Fig. 5) responded significantly more than the aCSF, 25 pmol/100 nl cocaine, or 100 mg% EtOH alone groups (Fig. 3). Individual lever $\times$ session ANOVAs conducted on these groups indicated lever discrimination in these cocaine + EtOH groups ($P < 0.05$). For example, in rats given the mixture of 75 mg% EtOH + 12.5 pmol/100 nl cocaine (Fig. 4, top right), there was a significant effect of lever ($F_{1,12} = 50.1$; $P < 0.001$) and a lever $\times$ session interaction ($F_{6,7} = 21.6$; $P < 0.001$). In this particular group, paired t tests between active and inactive lever responses indicated a significant discrimination

between active and inactive levers ($P < 0.007$) during sessions 1 through 4 and session 7.

To confirm that rats extinguished responding during aCSF substitution, the numbers of active lever responses and numbers of infusions self-administered among sessions 4 through 6 were compared. The results indicate that rats given the mixture of EtOH + cocaine reduced self-infusion behaviors during aCSF substitution. For example, in rats given the mixture of 75 mg% EtOH + 12.5 pmol/100 nl cocaine (Fig. 4, top right), paired t tests indicated that active lever responses and the number of self-infusions were significantly greater during the 4th session compared with session 5 or 6 ($P < 0.05$). Similar results were obtained in the other groups of rats that received more self-infusions than the aCSF group, with the exception of the group given the mixture of 100 mg% EtOH and 6.25 pmol/100 nl cocaine (Fig. 5, right top). In this group, substitution of aCSF did not reduce responding on the active lever during sessions 5 and 6 ($P > 0.05$).

The reinstatement of drug self-infusion was confirmed by examining the number of active lever responses and number of self-infusions during session 7 versus sessions 5 and 6. The results indicated that all groups, which received more self-infusions than the aCSF alone group during sessions 1 through 4, displayed reinstatement of responding when the original infusate was offered during session 7. For example, in the group receiving the mixture of 75 mg% EtOH + 12.5 pmol/100 nl cocaine (Fig. 4, top right), paired t tests indicated that active lever responses and the number of self-infusions were significantly greater during the 7th session compared with session 5 or 6 ($P < 0.05$). Similar results were obtained in the other groups of rats that received more self-infusions than the aCSF group.

Effects of ICS 205,930. Throughout the four acquisition sessions, rats readily self-infused the mixture of 75 mg% EtOH + 12.5 pmol/100 nl cocaine (32 ± 5 infusions/session) and responded significantly more on the active lever than inactive lever (all F values > 18.8 ; all $P < 0.001$; Fig. 6). Coadministration of 50 μ M ICS 205,930 in sessions 5 and 6 (Fig. 6, top) did not significantly alter responding on the active lever or the number of self-infusions ($P > 0.82$). How-

Fig. 6. Effects of ICS 205,930 on responding for the self-infusion of a mixture of 75 mg% EtOH and 12.5 pmol/100 nl cocaine into the posterior VTA of female Wistar rats ($n = 5-6$). For the first four sessions, a mixture of 75 mg% EtOH and 12.5 pmol/100 nl cocaine was given. In sessions 5 and 6, ICS 205,930 (50, 100, or 200 μ M) was coinfused with the EtOH and cocaine mixture. In session 7, the original infusate mixture was given. Data are the means $\pm$ S.E.M. *, $P < 0.05$, significantly higher responses on the active than inactive lever.

ever, coadministration of 100 (Fig. 6, middle) or 200 μ M (Fig. 6, bottom) ICS 205,930 in sessions 5 and 6 reduced the number of active lever responses and self-infusions ($P < 0.001$). When the original infusate was given during session 7, responding on the active lever and the number of self-infusions increased to levels observed in session 4 ($P > 0.67$).

Effects of LY278-584. Throughout the four acquisition sessions, rats readily self-infused 75 mg% EtOH + 12.5 pmol/100 nl cocaine (31 ± 7 infusions/session) and responded significantly more on the active than inactive lever ($P < 0.001$; Fig. 7). Coadministration of 25 μ M LY278-584 in sessions 5 and 6 (Fig. 7, top) did not significantly alter responding on the active lever or the number of self-infusions ($P > 0.77$). However, coadministration of 50 (Fig. 7, middle) or 100 μ M (Fig. 7, bottom) LY278-584 in sessions 5 and 6 reduced the number of active lever responses and self-infusions ($P < 0.001$). When the original infusate was given during session 7, responding on the active lever and the number of self-infusions increased to levels observed in session 4 ($P > 0.75$).

Discussion

The results of this study indicate that mixtures of sub-threshold concentrations of EtOH and cocaine can be self-infused into the posterior VTA (Figs. 2–5), as indicated by the

Fig. 7. Effects of LY278-584 on responding for the self-infusion of a mixture of 75 mg% EtOH and 12.5 pmol/100 nl cocaine into the posterior VTA of female Wistar rats ($n = 5-6$). For the first four sessions, a mixture of 75 mg% EtOH and 12.5 pmol/100 nl cocaine was given. In sessions 5 and 6, LY278-584 (25, 50, or 100 μ M) was coinfused with the EtOH and cocaine mixture. In session 7, the original infusate mixture was given. Data are the means $\pm$ S.E.M. *, $P < 0.05$, significantly higher responses on the active than inactive lever.

significantly higher numbers of infusions in these groups (e.g., 50 or 75 mg% EtOH + 12.5 or 25 pmol/100 nl cocaine; 100 mg% EtOH + 6.25, 12.5 or 25 pmol/100 nl cocaine) compared with aCSF or subthreshold concentration of EtOH or cocaine alone (Fig. 2). The coinfusion of a mixture of EtOH and cocaine into the posterior VTA did not seem to be a result of a general increase in behavioral activity because rats readily learned to discriminate the active from the inactive lever, extinguished self-infusion during aCSF substitution, and reinstated self-infusion when the original infusate was reintroduced (Figs. 4–5). These findings suggest that EtOH and cocaine produced synergistic reinforcing effects in the posterior VTA. Coadministration of a 5-HT₃ receptor antagonist reduced self-infusion of the mixture of EtOH and cocaine (Figs. 6 and 7), suggesting that the synergistic effects of EtOH and cocaine are mediated, at least in part, through activation of local 5-HT₃ receptors.

Previous studies indicated that EtOH, in the concentration range of 150 to 400 mg%, and cocaine, in the concentration range of 50 to 200 pmol/100 nl, were self-infused into the posterior VTA by female Wistar rats; the self-infusion of EtOH or cocaine was dependent on activation of local dopamine neurons (Rodd et al., 2004, 2005). Indeed, local application of high doses of EtOH (200 or 300 mg%) in the poste-

rior VTA stimulated dopamine neurons and increased terminal dopamine release (Ding et al., 2009a, 2011). However, local application of a lower dose of EtOH (100 mg%) only produced a small nonsignificant increase of dopamine release in the NAC shell (Ding et al., 2009a). This latter result suggests that subthreshold doses of EtOH may not produce sufficient stimulation of dopamine neurons to support self-infusion. This may also be the case for subthreshold doses of cocaine. It is possible that the mixture of subthreshold doses of cocaine and EtOH may produce sufficient stimulation of local dopamine neurons to forward the rewarding signal and support self-infusion of these drugs into the posterior VTA.

There are several possible mechanisms that might underlie the synergistic interactions of EtOH and cocaine in the posterior VTA: 1) EtOH and cocaine could be acting at the same site or sites, 2) EtOH and cocaine could be acting at different sites, 3) they could be acting at different parts of the same site or sites, and/or 4) a combination of a through c.

At the cellular level, evidence indicates that both EtOH and cocaine can act on VTA GABA interneurons. Systemic or local application of EtOH was shown to inhibit firing rates of VTA GABA neurons in vivo (Gallegos et al., 1999). A recent study demonstrated that 3 to 50 μ M cocaine reduced VTA GABA neuronal activity and GABA inhibitory postsynaptic transmission to VTA dopamine neurons (Steffensen et al., 2008). Different mechanisms seem to be involved in these effects of EtOH and cocaine. An inhibition of *N*-methyl-D-aspartate receptors may contribute to EtOH inhibition of GABA interneurons (Stobbs et al., 2004), whereas cocaine may attenuate the function of voltage-sensitive sodium channels to exert inhibition on VTA GABA neurons (Steffensen et al., 2008). Therefore, the mixture of cocaine and EtOH could augment inhibition of GABA interneurons, leading to enhanced disinhibition of VTA dopamine neurons.

At the molecular level, cocaine has high affinity for monoamine transporters, including the dopamine and 5-HT transporters. In general, cocaine can inhibit dopamine neuronal activity in the VTA by increasing extracellular dopamine, which in turn activates D2 autoreceptors in dopamine neurons (Bunney et al., 2000). However, cocaine at low doses was shown to potentiate EtOH-induced excitation of VTA dopamine neurons; the effect was reversed by blocking 5-HT₂ receptors (Bunney et al., 2000). It was proposed that cocaine at low doses would preferentially act at the 5-HT transporter due to its significantly higher affinity for the 5-HT transporter (Ritz et al., 1987). Therefore, it is possible that the synergistic interaction of cocaine and EtOH could be attributed to cocaine-induced increased synaptic levels of 5-HT, potentiating the action of EtOH at 5-HT₃ receptors. In support of this idea, Brodie et al. (1995) reported that 5-HT potentiated EtOH excitation of dopamine neurons in mid-brain slices. In addition, the effects of increased extracellular levels of 5-HT could be acting at other 5-HT receptors, e.g., 5-HT_{2A} (Bunney et al., 2000; Ding et al., 2009b), and, as a result, contribute to the synergistic interaction of cocaine and EtOH. The combination of the inhibitory actions of cocaine and EtOH on GABA interneurons and the inhibitory effects of cocaine on 5-HT reuptake, resulting in the enhanced actions of 5-HT on EtOH stimulation of 5-HT₃ receptors and the enhanced actions of 5-HT at 5-HT_{2A} receptors, may all

contribute to the synergistic interactions of cocaine and EtOH observed in the present study.

The current results demonstrated that coadministration of either ICS 205,930 or LY278-584 attenuated responding for the coinfusion of the mixture of EtOH and cocaine (Figs. 6 and 7). ICS 205,930 is a potent antagonist to the 5-HT₃ receptor and also possesses affinity for the 5-HT₄ receptor (Jin et al., 1999). However, LY278-584 is a highly selective 5-HT₃ receptor antagonist with little apparent affinity for other 5-HT receptors (Wong et al., 1989), suggesting that local 5-HT₄ receptors are not involved in the effects of ICS 205,930. In addition, ICS 205,930 was reported to have affinity for the GABA_A receptor (Klein et al., 1994). A recent study indicated that the stimulating effects of EtOH in the posterior VTA was not altered by a GABA_A receptor antagonist (Ding et al., 2011), suggesting that the effects of ICS 205,930 on EtOH self-administration may not be mediated by local GABA_A receptors. Evidence also shows that VTA GABA_A receptors are linked to self-administration of cocaine (Lee et al., 2007; Backes and Hemby, 2008). Although the involvement of VTA GABA_A receptors in the effects of ICS 205,930 on coinfusion of cocaine and EtOH cannot be excluded, there is no evidence showing an interaction between LY278-584 and the GABA_A receptor. All together, the evidence suggests that the effects of ICS 205,930 and LY278-584 observed in the current study were mainly mediated through antagonism of local 5-HT₃ receptors.

These results suggest that activation of local 5-HT₃ receptors is involved in mediating the synergistic reinforcing effects of cocaine and EtOH in the posterior VTA. These results extend previous findings demonstrating that activation of local 5-HT₃ receptors was involved in the reinforcing effects of either EtOH or cocaine in the posterior VTA (Rodd-Henricks et al., 2003; Rodd et al., 2005). The findings are also in line with the study showing that coadministration of ICS 205,930 attenuates the stimulating effects of EtOH on mesocorticolimbic dopamine neurons within the posterior VTA (Ding et al., 2011).

A moderate density of 5-HT₃ receptors is present in the VTA (Ge et al., 1997). Although the cellular localization of these receptors remains unknown, activation of 5-HT₃ receptors in the posterior VTA produces a stimulatory net effect on local dopamine neurons (Liu et al., 2006). A previous electrophysiological study indicated that EtOH at doses of 1 to 25 mM (approximately 5–110 mg%) induced a 5 to 20% increase of 5-HT₃ receptor-mediated ion currents in vitro (Lovinger and White, 1991). Cocaine at low doses can inhibit serotonin transporters and increase extracellular 5-HT levels, which in turn can activate postsynaptic 5-HT receptors, including 5-HT₃ receptors (Bunney et al., 2000, 2001). Therefore, the two 5-HT₃ receptor antagonists can block the indirect effects of cocaine on dopamine neurons in addition to the direct antagonism of the effects of EtOH on 5-HT₃ receptors. The net effects would be an attenuation of dopamine excitation induced by cocaine and EtOH, leading to reduced self-infusion behavior.

Given the 5-HT mechanism of the action of cocaine, it is possible that other 5-HT receptors in addition to the 5-HT₃ receptor may also be involved in the synergistic effects of cocaine and EtOH, e.g., the 5-HT_{2A} receptor. The cocaine-induced potentiation of EtOH excitation of VTA dopamine neurons was prevented by a 5-HT_{2A} receptor antagonist

(Bunney et al., 2000). In addition, the self-administration of EtOH in the posterior VTA was attenuated by coinfusion of a selective 5-HT_{2A} receptor antagonist (Ding et al., 2009b). Therefore, it may be noteworthy to examine the involvement of local 5-HT_{2A} receptors in the synergistic effects of EtOH and cocaine.

Overall, the present data indicated that cocaine and EtOH produced synergistic effects at subthreshold doses in the posterior VTA. These findings suggest that the increased sensitivity of the posterior VTA to the mixture of cocaine and EtOH may be a factor contributing to coabuse of alcohol and cocaine. In addition, the synergistic effects of EtOH and cocaine seemed to be mediated, at least in part, by activation of 5-HT₃ receptors in the posterior VTA. These findings may provide important information in identifying pharmacological targets for the development of therapeutics to reduce alcohol and cocaine coabuse.

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Authorship Contributions

Participated in research design: Ding, Oster, McBride, and Rodd.

Conducted experiments: Ding, Oster, Hauser, Toalston, Bell, and Rodd.

Performed data analysis: Ding, Oster, and Rodd.

Wrote or contributed to the writing of the manuscript: Ding, McBride, and Rodd.

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