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14. ABSTRACT

The experiments were carried out in 5-HT1B receptor knock-out (KO) mice and wild-type (WT) mice. One-probe microdialysis was performed. In the first set of experiments, saline or RU 24969 (0.5 and 1 mg/kg), a 5-HT1B/1A receptor agonist, was injected ip to the KO or WT mice. The results showed that administration of RU 24969 at the dose of 1 mg/kg significantly increased dopamine (DA) concentrations in the nucleus accumbens (NACC) of the WT mice but not the KO mice. In the second set of experiments, saline or ethanol (1 and 2 g/kg) was injected ip to the KO or WT mice. The results showed that administration of ethanol at the dose of 2 g/kg produced more pronounced increases in the NACC DA in the WT mice than in the KO mice. In the third set of experiments, RU 24969 (0.5 mg/kg) was injected ip to KO or WT mice. Twenty min later, each mouse received ethanol injections at the dose of 1 or 2 g/kg. The results showed that pretreatment with RU 24969 enhanced the effects of ethanol on NACC DA in the WT mice but not in the KO mice. These results obtained from transgenic animals provide additional support to the hypothesis that 5-HT1B receptors are partially involved in ethanol-induced increases in mesolimbic DA transmission.

15. SUBJECT TERMS
Ethanol, Dopamine, Serotonin, 5-HT1B receptor, Nucleus accumbens, Knockout mice, Microdialysis

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INTRODUCTION

The purpose of this project entitled "Ethanol and mesolimbic serotonin (5-HT)/dopamine (DA) interactions via 5-HT-1B receptors" is to investigate whether activation of 5-HT-1B receptors within the ventral tegmental area (VTA) facilitates DA transmission in the ipsilateral nucleus accumbens (NACC) and potentiates ethanol-induced increases in NACC DA by 5-HT-1B receptor-mediated GABA mechanisms. The scope of this project covers the following specific aims: (1) To determine the involvement of 5-HT-1B heteroreceptors on GABA terminals in the VTA in the modulation of GABA release in the VTA and DA release in the ipsilateral NACC, and its involvement in the neurochemical effect of acute ethanol in freely moving animals; (2) To compare the impact of 5-HT-1B receptor activation on DA transmission in the NACC and on ethanol's neurochemical effects between 5-HT-1B receptor knock-out (KO) mice and their counterparts wild-type (WT) mice, (3) To determine the involvement of 5-HT-1B heteroreceptors on GABA terminals in the VTA in the modulation of DA and GABA releases in the VTA, and its involvement in the effect of ethanol in superfused VTA slices.

BODY

Specific Aim 1: To determine the involvement of 5-HT-1B heteroreceptors on GABA terminals in the VTA in the modulation of GABA release in the VTA and DA release in the ipsilateral NACC, and its involvement in the neurochemical effect of acute ethanol in freely moving animals

There are two hypotheses under Specific Aim 1: (1) Activation of 5-HT-1B receptors in the VTA decreases GABA release in this area and increases DA transmission in the ipsilateral NACC and (2) Activation and blockade of VTA 5-HT-1B receptors potentiates and attenuates ethanol's effects on DA transmission in the ipsilateral NACC, respectively. According to Statement of Work, experiments under Specific Aim 1 are to be completed in two years.

The experiments related to Specific Aim 1 have been completed. The data have generated two papers that have been published in *Brain Research* (1021: 82-91, 2004 and 1060: 126-137, 2005). The reprint of the paper published in 2004 has already been submitted as the attachment of the last annual report. The paper published in 2005 is attached here with the present report (please see the Appendix).

Specific Aim 2: To compare the impact of 5-HT-1B receptor activation on DA transmission in the NACC and on ethanol's neurochemical effects between 5-HT-1B receptor knock-out (KO) mice and their counterparts wild-type (WT) mice

There are three hypotheses under Specific Aim 2: (1) Facilitation of NACC DA transmission by activation of the 5-HT-1B receptor is absent in 5-HT-1B receptor knock-out mice; (2) Systemic ethanol-induced increases in NACC DA is more pronounced in WT mice than in KO mice; and (3) Potentiation of the effects of ethanol on NACC DA by activation of the 5-HT-1B receptor is absent in 5-HT-1B receptor knock-out mice.

According to "Statement of Work", the specific aim 2 would be completed in 18 months (Year 3 and the first half of Year 4). However, as stated in the previous reports, the mouse hepatitis virus spread at the vivarium of Dr. Rene Hen, a professor of Columbia University, who had a KO mouse colony and originally promised me to provide the KO mice that are required for the whole project. Due to this unexpected situation, I cannot obtain these animals directly from Dr. Hen. As a result, it was necessary for me to obtain the KO breeder from other sources, and then breed and genotype these animals at my own laboratories. However, breeding and genotyping substantially increased our workload, and significantly delayed the progress of the project. Now, my application for a one-year no-cost extension of the project has been approved. The following data reported here were obtained during Year 4 and are related to the specific aim 2.

1. Comparisons of the effects of RU 24969 on extracellular concentrations of DA in the NACC between the KO and WT mice. These experiments were performed to address Hypothesis 1 under the specific aim 2. Please note that the experiments related to 1 mg/kg of RU 24969 were done during Year 3 and have already been reported in the last annual report. But, the

experiments related to 0.5 mg/kg of RU 24969 were accomplished during Year 4 and the data are new. In order to give a complete picture regarding the effects of RU 24969 on NACC DA in the KO and WT mice, the data with both doses are put together and reported as follows.

One-probe microdialysis was employed. Guide cannulae manufactured by CMA Microdialysis Co. were stereotaxically implanted above the NACC of the mice under anesthesia. Seven days later, a CMA/7 microdialysis probe (1 mm long) was inserted and dialysis was performed in a freely-moving condition. After basal DA in the NACC was stable, saline or RU 24969 (0.5 and 1 mg/kg), a 5-HT-1B/1A receptor agonist, was injected ip to KO or WT mice. Extracellular DA concentrations in the NACC after saline or RU 24969 administration were measured. The basal DA levels (fmol/sample) in the NACC were: 17.05 ± 2.99 (the KO saline group, n=6), 14.79 ± 1.9 (the KO RU 24969 0.5 mg group, n=6), 18.62 ± 2.03 (the KO RU 24969 1 mg group, n=6), 16.93 ± 2.53 (the WT saline group, n=6), 16.01 ± 1.62 (the WT RU 24969 0.5 mg group, n=6), and 17.45 ± 2.43 (the WT RU 24969 1 mg group, n=5). There were no statistically significant differences in the basal DA values between the KO and WT mice, nor were there differences among the groups in the KO or WT mice.

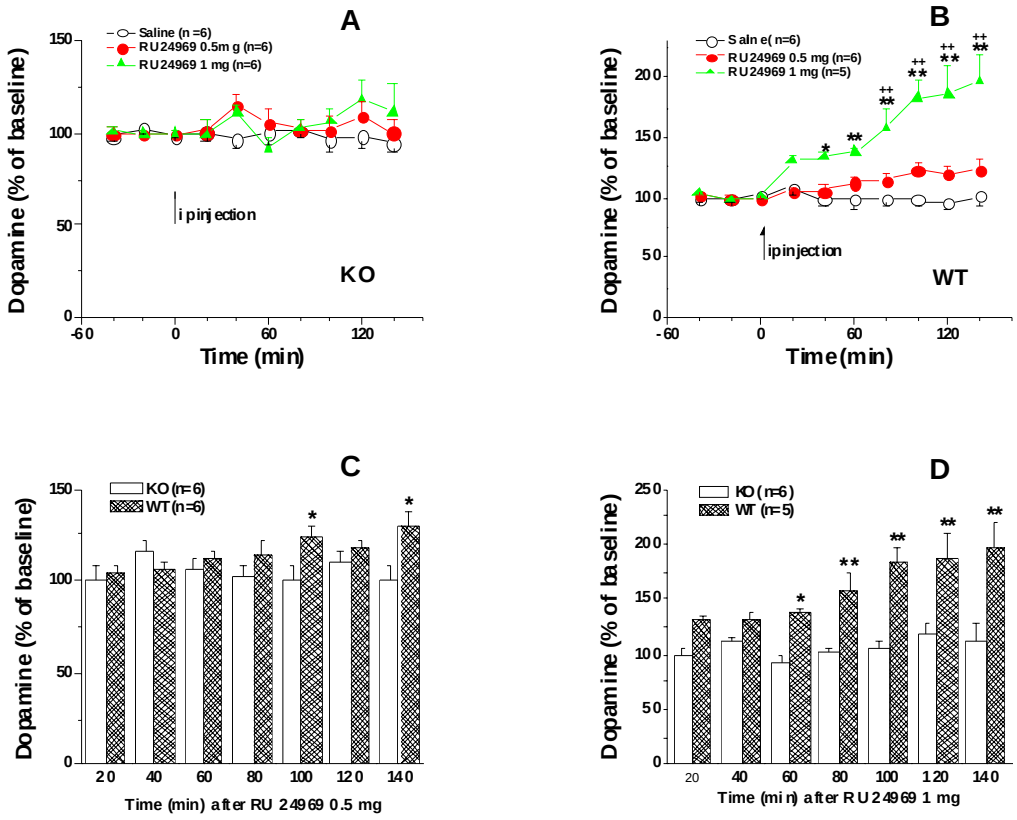

Fig 1. The effects of RU 24969 on extracellular DA concentrations in the NACC of KO and WT mice. Panels A and B show the time courses of NACC DA after administration of RU 24969 in the KO and WT mice, respectively. RU 24969 (0.5 or1 mg/kg) or saline was injected ip as indicated by the arrow. Results are mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$ as compared with the saline group; $^{++}$ $P < 0.01$ as compared with the RU 24969 0.5 mg group (two-way ANOVA followed by Tukey's tests). Panels C and D shows comparisons of NACC DA between the KO and WT mice in response to injections of RU 24969 at the doses of 0.5 and 1 mg/kg, respectively. * $P < 0.05$, ** $P < 0.01$ as compared with the KO mice (two-way ANOVA followed by Tukey's tests).

As shown in Fig 1, administration of RU 24969 at the doses of 0.5 and 1 mg/kg did not produce significant changes in extracellular NACC DA in the KO mice as compared with the saline group (Panel A). In contrast, administration of RU 24969 at the same doses produced a dose-related increase in extracellular NACC DA in the WT mice (Panel B). There was a slight increase in NACC DA in the WT mice after 0.5 mg/kg of RU 24969 although the effect did not reach statistical significances. After administration of RU 24969 at the higher dose (1 mg/kg), however, extracellular NACC DA levels were significantly higher than those in response to administration of either saline or 0.5 mg/kg of RU 24969 from 40 min until the end of the experiment ($P < 0.05$ or $P < 0.01$, two-way ANOVA followed by Tukey tests). Panels C and D show comparisons of NACC DA between the KO and WT mice following injections of RU 24969 at the doses of 0.5 and 1 mg/kg, respectively. From these figures we can see that RU 24969 increased NACC DA in the WT but not in the 5-HT1B receptor KO mice. Together with those obtained from the previous study showing that activation of 5-HT-1B receptors within the VTA increases DA release in the ipsilateral NACC in rats[1], these data support the notion that activation of 5-HT-1B receptors facilitates DA transmission in the mesolimbic DA pathway.

Since RU 24969 is a 5-HT-1B/1A receptor agonist, a role of 5-HT1A receptors in the observed effect of RU 24969 on NACC DA in the WT mice cannot be ruled out. However, studies by Ichikawa and Meltzer[2,3] do not support the involvement of 5-HT1A receptor subtypes. They demonstrated that systemic administration of 8-OH-DPAT, a selective 5-HT1A receptor agonist, either had no effects on (at the dose of 0.05 mg/kg)[2] or decreased (at the dose of 0.2 mg/kg)[3] NACC DA release, suggesting that activation of 5-HT1A receptors may not be associated with facilitation of NACC DA release. However, the possible involvement of 5-HT1A receptors in RU 24969's effects still needs assessment. These experiments are currently in progress.

2. Comparisons of NACC DA in response to systemic ethanol between KO and WT mice. These experiments were performed to address Hypothesis 2 under the specific aim 2 and completed during Year 4.

One-probe microdialysis was employed as described above. After basal DA in the NACC was stable, saline or ethanol at the dose of 1 or 2 g/kg, was injected ip to KO or WT mice. Extracellular DA concentrations in the NACC after saline or ethanol were measured. The basal DA levels (fmol/sample) in the NACC were: 13.72 ± 2.28 (the KO saline group, n=6), 15.08 ± 1.65 (the KO ethanol 1 g/kg group, n=7), 12.02 ± 1.12 (the KO ethanol 2 g/kg group, n=7), 13.6 ± 2.11 (the WT saline group, n=6), 14.09 ± 1.47 (the WT ethanol 1 g/kg group, n=8), and 13.25

± 1.61 (the WT ethanol 2 g/kg group, n=7). There were no statistically significant differences in the basal DA values between the KO and WT mice, nor were there differences among the groups in KO or WT mice.

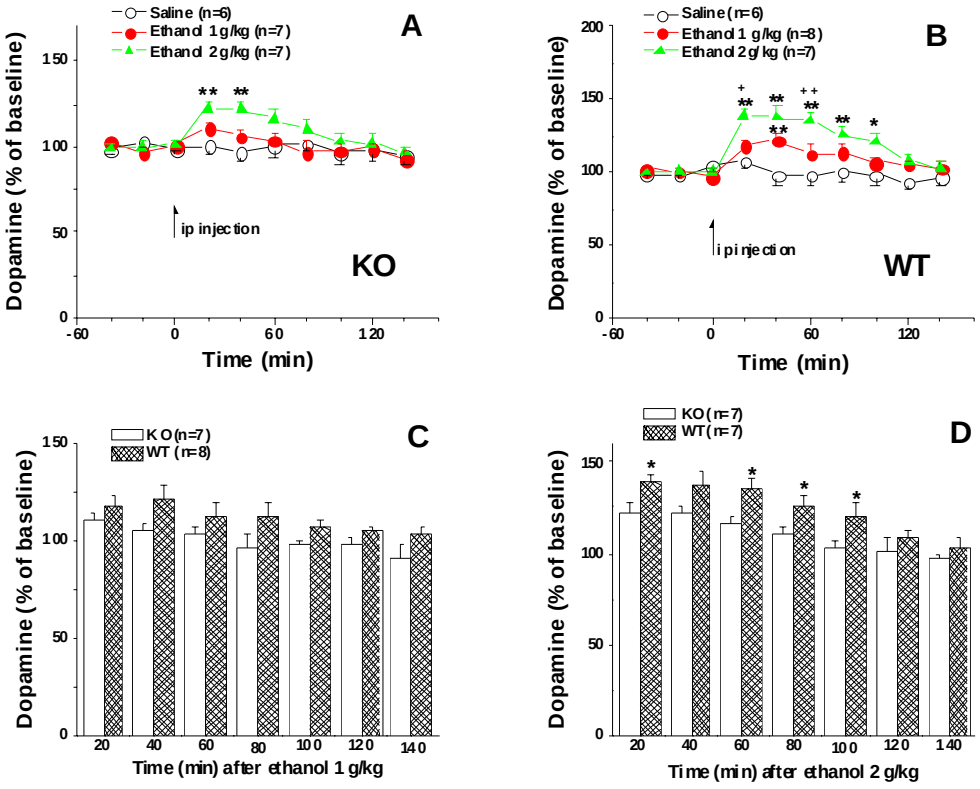

Fig 2. Effects of ethanol on NACC DA in the KO and WT mice. After basal NACC DA was stable, saline or ethanol (1 and 2 g/kg) was injected ip as indicated by the arrow. Results are mean ± S.E.M. Panels A and B show the time courses of extracellular DA in the NACC following saline or ethanol injections in the KO and WT mice, respectively. ***** $P < 0.05$; ****** $P < 0.01$ as compared with the saline group. $^{+}$ $P < 0.05$; $^{++}$ $P < 0.01$ as compared with the 1 g/kg ethanol group (two-way ANOVA followed by Tukey tests). Panels C and D show comparisons of NACC DA between the KO and WT mice in response to ethanol at the doses of 1 and 2 g/kg, respectively. ***** $P < 0.05$ as compared with the KO mice (two-way ANOVA followed by Tukey tests).

As shown in Fig 2, significant increases in NACC DA in the KO mice were observed only after administration of the higher dose (2 g/kg) of ethanol. The lower dose (1 g/kg) of ethanol caused a slight but not statistically significant increase in the KO mice (Panel A). However, administration of the same doses of ethanol all significantly increased NACC DA in the WT mice (panel B). Comparisons of the NACC DA responses after 1 g/kg of ethanol show that there are no significant differences in any time points after ethanol between the KO and WT mice (Panel C). However, administration of ethanol at the dose of 2 g/kg produced more pronounced increases of NACC DA in the WT mice than in the KO mice. Statistical analyses show that significant differences in NACC DA after 2 g/kg of ethanol were seen at the time points of 20, 60, 80, and 100 min after ethanol administration between the WT and KO mice (Panel D).

The results indicate that systemic administration of ethanol at the dose of 2 g/kg results in more pronounced increases in NACC DA in the WT mice than in the KO mice. The present study, carried out in the transgenic animal that lacks 5-HT1B receptors, validates the results obtained with the classical pharmacological approach in our previous study showing that activation and blockade of VTA 5-HT1B receptors potentiate and attenuate the ethanol-induced increases in rat mesolimbic DA transmission[8]. Taken together, the data suggest that 5-HT1B receptors may be partially involved in ethanol-stimulated NACC DA release.

3. Effects of pretreatment with the 5-HT1B/1A receptor agonist RU 24969 on ethanol-induced increases of NACC DA release in the KO and WT mice. These experiments were carried out to address Hypothesis 3 under the specific aim 2 and completed during Year 4.

One-probe microdialysis was performed as described above. After basal DA in the NACC was stable, RU 24969 was injected ip to the KO and WT mice, respectively. Twenty minutes later, each mouse received an ethanol injection (1 or 2 g/kg, ip) and extracellular DA in the NACC was monitored. Extracellular DA concentrations in the NACC after ethanol in the presence of the pretreatment with RU 24969 (this experiment) was compared with those in the absence of RU 24969 (the above experiment). The basal NACC DA levels (fmol/sample) in the KO mice were: 11.01 ± 1.44 (the group of RU 24969 + ethanol 1 g/kg, n=7) and 11.88 ± 1.40 (the group of RU 24969 + ethanol 2 g/kg, n=8); in the WT mice were: 10.96 ± 2.34 (the group of RU 24969 + ethanol 1 g/kg, n=7) and 12.46 ± 2.11 (the group of RU 24969 + ethanol 2 g/kg, n=7).

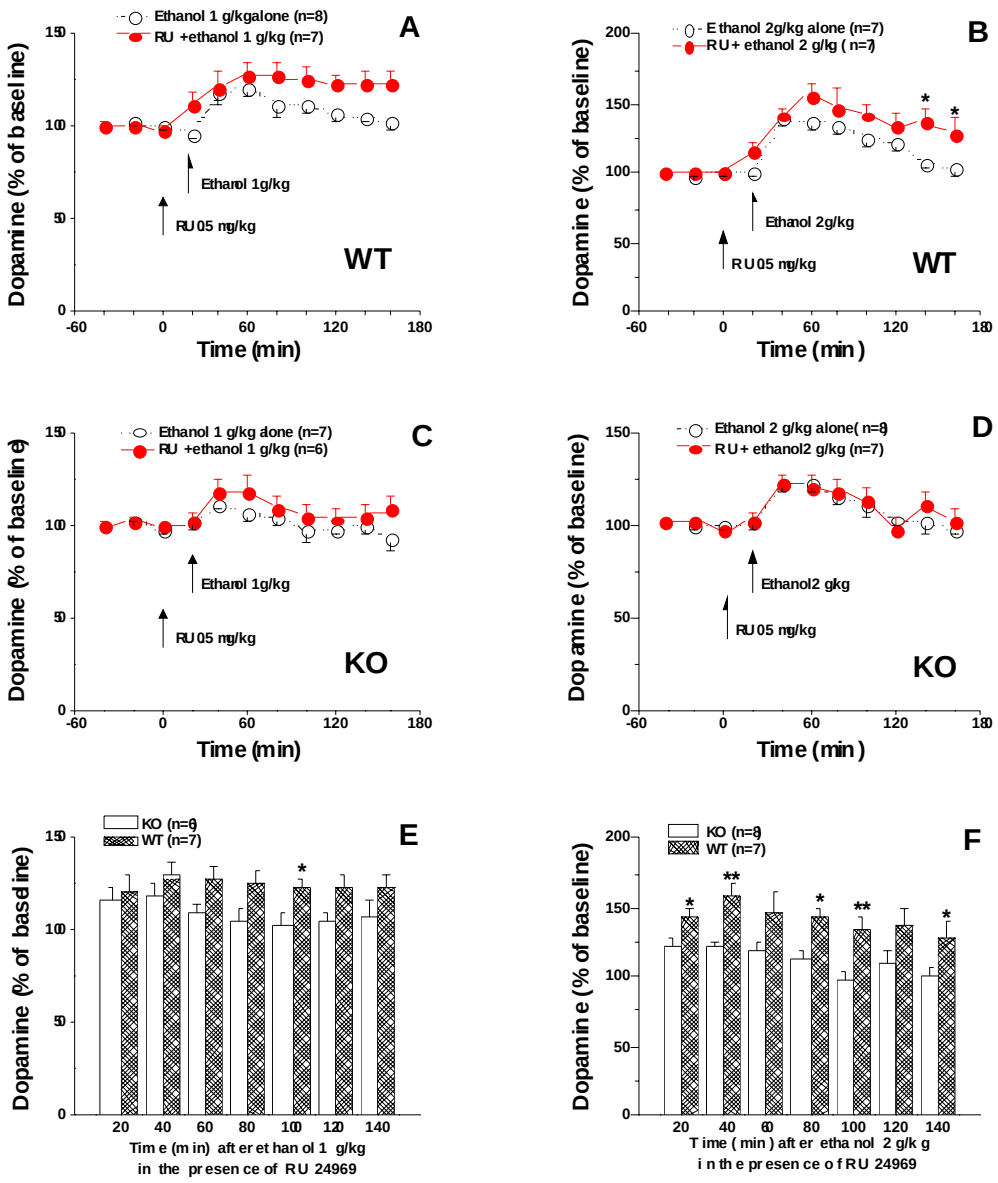

Fig 3. Effects of pretreatment with RU 24969 on ethanol (1 and 2 g/kg)-induced NACC DA release in the KO and WT mice. RU 24969 (0.5 mg/kg) was injected ip as indicated by the arrow. Twenty min later, ethanol (1 or 2 g/kg) was administered ip indicated by another arrow. The results are mean ± S.E.M. The data of the ethanol alone groups were obtained from Fig 2.

Panels A, B, C, and D show the time courses of extracellular DA in the NACC after administration of RU 24969 and ethanol in the KO or WT mice. ***** $P < 0.05$ as compared with the ethanol alone group (two-way ANOVA followed by *t* tests). Panels E and F show comparisons of extracellular NACC DA after administration of ethanol at the doses of 1 (Panel E) and 2 g/kg (Panel F) in the presence of RU 24969 between the KO and WT mice. ***** $P < 0.05$, ****** $P < 0.01$ as compared with the KO mice (two-way ANOVA followed by t tests).

Fig 3 shows the effects of pretreatment with RU 24969 on the ethanol-induced increase of NACC DA in the KO and WT mice. As shown in Panels C and D of this figure, administration of RU 24969 did not significantly alter the effects of ethanol in the KO mice since extracellular DA concentrations were similar after administration of ethanol at the doses of 1 and 2 g/kg between the ethanol alone group and the ethanol plus RU 24969 group. In the WT mice, pretreatment with RU 24969 did not significantly alter the effects of 1 g/kg of ethanol (panel A), but did enhance the effects of 2 g/kg of ethanol on NACC DA (Panel B). In the absence of RU 24969, extracellular DA in the NACC of the WT mice increased to the maximum level after administration of ethanol at the dose of 2 g/kg, then declined gradually and reached the baseline level at 120 min after ethanol injection (Panel B of Fig 2). In contrast, in the presence of RU 24969, extracellular DA still remained at significantly high levels as compared with the ethanol alone group at 120-140 min after ethanol administration (panel B of this figure). The results indicated that the pretreatment with RU 24969 significantly enhanced the effects of ethanol on NACC DA in the WT mice, especially when ethanol was used at higher dose.

The results presented in Fig 1 show that administration of RU 24969 at the dose of 0.5 mg/kg did not significantly alter NACC DA in the WT mice although there was a slight and non-significant increase after drug administration. This result suggests that this dose of RU 24969 may not cause sufficient activation of 5-HT_{1B} receptors to produce significant increases in the activity of mesolimbic DA neurons under the present experimental conditions. However, this dose of RU 24969 did enhance the effects of 2 g/kg of ethanol on extracellular DA in the NACC of WT mice. The present data show that the pretreatment with RU 24969 significantly prolongs the effects of ethanol although it does not significantly enhance ethanol's peak effects. As shown in panel B of Fig 3, extracellular DA remains significantly high at 120 – 140 min after ethanol in the presence of RU 24969 as compared with that in the absence of RU 24969. It was reported that the blood alcohol concentration (BAC) rose progressively to a peak at 20-40 minutes and then decreased thereafter, which was temporally correlated with changes of extracellular DA and 5-HT[4,5], following intraperitoneal injection of 1 - 2 g/kg of ethanol[6,7,]. Therefore, our observation suggests that enhancement of ethanol's effects by RU 24969 occurs mainly during the descending limb of the BAC. Although the precise reasons for this type of enhancement are not presently understood, it is conceivable that this phenomenon may be due to additive or synergetic activation of 5-HT1B receptors by RU 24969 and released 5-HT by ethanol. The observed lack of further increases of ethanol's peak effects by RU 24969 may be a result of the 5-HT1B receptors already being maximally (or near maximally) activated by ethanol-evoked 5-HT, consequently, leading to little potential for further activation by RU 24969. The present data showing enhancement of NACC DA by activation of 5-HT1B receptors in the WT mice but not in the 5-HT1B receptor KO mice provide additional support to the suggestion that activation of

5-HT1B receptors may contribute to systemic ethanol-induced increases of mesolimbic DA neuronal activities.

KEY RESEARCH ACCOMPLISHMENTS

We found the followings:

1. Administration of RU 24969 (1 mg/kg, ip), a 5-HT1B/1A receptor agonist, increased NACC DA concentrations in the WT mice but not in the 5-HT1B receptor KO mice.
2. Systemic administration of ethanol at the dose of 2 g/kg produced more pronounced increases in the NACC DA concentrations in the WT mice than in the KO mice.
3. Pretreatment of RU 24969 enhanced the effects of subsequent administration of ethanol on NACC DA in the WT mice but not in the 5-HT1B receptor KO mice.

REPORTABLE OUTCOMES

Yan QS, Zheng SZ, Feng MJ, Yan SE,: Involvement of 5-HT_{1B} receptors within the ventral tegmental area in ethanol-induced increases in mesolimbic dopaminergic transmission. *Brain Research*, 1060:126-137, 2005 (please see Appendixes).

CONCLUSION

The results show that administration of RU 24969, a 5-HT1B/1A receptor agonist, increased NACC DA concentrations in the WT mice but not in the 5-HT1B receptor KO mice. These results are consistent with the hypothesis that facilitation of NACC DA transmission by activation of the 5-HT1B receptor is absent in 5-HT1B receptor knock-out (KO) mice. However, further experiments with a selective 5-HT1B receptor antagonist such as SB 216641 are required to strengthen this conclusion. The future experiments will be performed to address whether RU 24969-induced increases in NACC DA concentrations in the WT mice are inhibited by SB 216641.

The results also show that systemic administration of ethanol produced more pronounced increases in NACC DA concentrations in the WT mice than in the KO mice. Since the KO mice lack 5-HT1B receptors, the data are consistent with the involvement of 5-HT1B receptors in ethanol-stimulated NACC DA release. This hypothesis is further supported by the findings showing that pretreatment with RU 24969 enhanced ethanol's effects on NACC DA in the WT but not in the KO mice. These data obtained from the transgenic animals extend and confirm the previous studies obtained with the pharmacological approach, and support the hypothesis that 5-HT1B receptors are partially involved in ethanol-induced increases in mesolimbic DA transmission.

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APPENDIX

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BRAIN RESEARCH

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Research Report

Involvement of 5-HT_{1B} receptors within the ventral tegmental area in ethanol-induced increases in mesolimbic dopaminergic transmission

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Abstract

Evidence suggests that 5-hydroxytriptamine-1B (5-HT_{1B}) receptors play a role in modifying ethanol's reinforcing effects and voluntary intake, and that 5-HT_{1B} receptors within the ventral tegmental area (VTA) are involved in regulation of mesolimbic dopaminergic neuronal activity. Since increased mesolimbic dopaminergic transmission has been implicated in ethanol's reinforcing properties, this study was designed to assess the involvement of VTA 5-HT_{1B} receptors in mediating the stimulatory effects of ethanol on VTA dopaminergic neurons. Dual-probe microdialysis was performed in freely moving adult Sprague–Dawley rats with one probe within the VTA and the other within the ipsilateral nucleus accumbens (NACC). Dopamine (DA) levels in dialysates from both areas, as the index of the activity of mesolimbic DA neurons, were measured simultaneously. The results showed that intraperitoneal injection of ethanol at the doses of 1 and 2 g/kg increased extracellular DA concentrations in both the VTA and the NACC, suggesting increased DA neuronal activity. These ethanol-induced increases of the DA release in the VTA and the NACC were significantly attenuated by intra-tegmental infusion of SB 216641 (a 5-HT_{1B} receptor antagonist), but not BRL 15572 (a 5-$HT_{1D/1A}$ receptor antagonist) or WAY 100635 (a 5-HT_{1A} receptor antagonist). Administration of ethanol at the same doses did not significantly alter extracellular levels of GABA in the VTA. The results also showed that intra-tegmental infusion of CP 94253, a 5-HT_{1B} receptor agonist, significantly prolonged the effects of ethanol on NACC DA. The results suggest that blockade and activation of VTA 5-HT_{1B} receptors attenuates and potentiates the neurochemical effects of ethanol, respectively, and support the suggestion that VTA 5-HT_{1B} receptors may be involved in part in mediating the activating effects of ethanol on mesolimbic DA neurons.

Theme: Receptors
Topic: Serotonin receptor

Keywords: Ventral tegmental area; Nucleus accumbens; Ethanol; Dopamine; 5-HT_{1B} receptor; Dual-probe microdialysis

1. Introduction

It has been suggested that 5-hydroxytryptamine-1B (serotonin, 5-HT_{1B}) receptors play a role in modifying the reinforcing [15,43,53], intoxicating [15], and discriminative stimulus effects of ethanol [23,28] as well as regulating its voluntary intake [15,43,44]. Evidence supporting the role of 5-HT_{1B} receptors in mediating ethanol's effects comes from various studies. McBride et al. [44] investigated the densities of subtypes of 5-HT and dopamine (DA) receptors in the central nervous system (CNS) of alcohol-naïve alcohol-preferring P and alcohol non-preferring NP lines of rats. They found that there were no significant differences in the regional CNS densities of D_1, D_3, or 5-HT_3 binding sites between the P and NP lines. However, lower densities of 5-HT_{1B} receptors were found in the cortex, the septum, and the amygdala of the P lines compared with the NP lines. These results suggest that an innate alteration in the densities of 5-HT_{1B} receptors may be associated with the disparate alcohol-drinking behaviors of these two lines. Moreover, mice lacking the 5-HT_{1B} receptor gene consume more alcohol than wild-type mice and are more sensitive to some of ataxic

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effects of alcohol ([4,15], but see [17,27]). Using quantitative trait loci analysis in BXD recombinant inbred mice, the 5-HT_{1B} receptor gene has been identified as a potential candidate gene for alcohol preference [16]. Furthermore, polymorphisms in the 5-HT_{1B} receptor gene have been linked to antisocial personality and alcoholism in human ([39], but see [32]). The data cited above suggest that 5-HT_{1B} receptors might be one of the components in the genetic background underlying alcohol preference under some circumstances in rodents although recent research efforts have not consistently confirmed these original findings [17,27].

The involvement of 5-HT_{1B} receptors in alcohol-drinking behavior is further supported by the observations showing decreased alcohol consumption by 5-HT_{1B} agonists. Higgins et al. [30] reported that in rats that have been maintained on continuous access drinking, mCPP (a 5-HT_{1B} receptor agonist) suppressed alcohol intake during the first hour post-injection. In limited access drinking, mCPP produced dose-dependent suppression of alcohol intake in the rat. Wilson et al. [60] have tested the effects of TFMPP, a 5-$HT_{1B/2C/1A}$ receptor agonist [13], on ethanol ingestion and maintained behavior in an operant self-administration paradigm. They found that TFMPP produced a reduction in ethanol ingestion and maintained behavior at doses that failed to reduce locomotor activity. These results were consistent with the concept that activation of 5-HT_{1B} receptors may reduce ethanol intake and reinforced behavior. However, the contribution of 5-HT_{1A} and/or 5-HT_{2C} receptors to the TFMPP's actions cannot be ruled out in Wilson et al.'s study [60]. Recently, Tomkins and O'Neill [57] further explored the role of 5-HT_{1B} receptors in regulation of ethanol intake. They examined influences of the 5-$HT_{1B/1A}$ receptor agonist RU 24969 or CGS 12066B alone, RU 24969 plus GR 127935 (a 5-$HT_{1B/1D}$ receptor antagonists), and RU 24969 plus WAY 100135 or WAY 100635 (5-HT_{1A} receptor antagonists) on oral ethanol self-administration, respectively. The results showed that both RU 24969 and CGS 12066B significantly suppressed oral ethanol self-administration. Administration of GR 127935 significantly reversed the effects elicited by RU 24969, whereas neither WAY 100635 nor WAY 100135 had any effects. These data demonstrated that 5-HT_{1B} receptor activation suppressed oral ethanol self-administration and further supported the hypothesis that 5-HT_{1B} receptors play a role in regulating ethanol intake. Recently, 5-HT_{1B} receptors have also been found to be involved in mediation of the conditioned, or secondary reinforcing properties of ethanol when ethanol-paired conditioned stimulus paradigms were used [61].

The neurochemical mechanisms involved in the modulation of the alcohol-drinking behaviors by 5-HT_{1B} receptors are not known. Several lines of evidence suggest that DA neurons in the ventral tegmental area (VTA) are important for ethanol's reinforcing effects and self-administration. Thus, administration of ethanol increased the firing of DA neurons in the VTA [7,8,25] and enhanced somatodendritic DA release in the VTA [10] and terminal DA release in the nucleus accumbens (NACC) [26,59,63]. Moreover, alcohol-preferring P rats and Wistar rats self-administered ethanol directly into the VTA [24,54]. Previous studies also showed that VTA 5-HT_{1B} receptors may be involved in the modulation of activity of the mesolimbic DA neurons. Activation of VTA 5-HT_{1B} receptors has been reported to increase DA release in both the VTA and the ipsilateral NACC [64,67]. Therefore, a dopaminergic mechanism may be involved in 5-HT_{1B} receptor-mediated regulation of the alcohol-drinking behavior. That is, the reported suppression of alcohol intakes by 5-HT_{1B} receptor agonists may be due to the 5-HT_{1B} receptor activation-induced potentiation of ethanol's actions on mesolimbic DA neurons, therefore leading to lesser amounts of alcohol being consumed to produce the same CNS effects. The present study was designed to assess the involvement of VTA 5-HT_{1B} receptors in ethanol-induced increases in mesolimbic DA transmission. Towards this aim, dual-probe microdialysis was used with one in the VTA and the other in the ipsilateral nucleus accumbens (NACC) of freely moving Sprague–Dawley rats. Several serotonergic agents were administered into the VTA via retrograde microdialysis to minimize the effects of the compounds on the structures other than the VTA. The effects of the serotonergic manipulations on ethanol-induced increases of extracellular DA levels in the VTA and the NACC were assessed simultaneously. Since previous studies suggest that 5-HT_{1B} receptor-mediated activation of mesolimbic DA neurons may involve VTA GABAergic neurotransmission [67], extracellular GABA concentrations in the VTA were also monitored in some experiments to see whether ethanol affects VTA GABA levels as well.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 250–300 g at the time of surgery, were obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN, USA). They were housed at 21 ± 3 °C, 40–60% relative humidity, and maintained under 12:12-h light–dark conditions with ad libitum access to food and water. All animal care and experimentation were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Illinois College of Medicine at Peoria.

2.2. Drugs

WAY-100635 maleate {*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-2-pyridinyl-cyclohexanecarboxamide maleate}, BRL 15572 {4-[3-chlorophenyl]-α-[diphenyl-

methyl]-1-piperazineethanol hydrochloride} were purchased from Sigma (St. Louis, MO, USA). SB 216641 hydrochloride {*N*-[3-[3-(dimethylamino)ethoxy]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1′-biphenyl]-4-carboxamide hydrochloride} was obtained from Tocris (Ellisville, MO, USA). CP 94253 {5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo [3,2-b]pyridine} was generously provided by Pfizer (Groton, CT, USA). All drugs were dissolved in water and then diluted to desired concentrations with artificial cerebrospinal fluid (ACSF) before administration. Ethanol (Sigma) was administered intraperitoneally in a 20% solution (w/v) with 0.9% sterile saline. Other reagents used were of analytical grade.

2.3. Microdialysis

The animals were prepared for the microdialysis experiments as described in a previous paper [67]. In brief, surgery was conducted on a Kopf stereotaxic instrument under anesthesia with a combination of sodium pentobarbital (35 mg/kg ip) and halothane (5% in oxygen). Dialysis guide cannulae (Harvard Apparatus, Inc., S. Natick, MA, USA) were stereotaxically implanted over both the VTA and the ipsilateral NACC and attached to the skull with dental acrylic and machine screws. The coordinates relative to bregma and skull surface were as follows: the VTA: AP −5.2 mm, L 3 mm (at an angle of 14° from the sagittal plane to avoid rupture of the sagittal sinus), DV 8.0 mm, and the NACC: AP 1.7 mm, L 1.4 mm, DV 8.0 mm according to the Atlas of Paxinos and Watson [50]. The period of post-surgical recovery was at least 5 days. On the evening before the experimental day, each rat was placed in a Plexiglas chamber and dialysis probes (1 and 2 mm in length for the VTA and NACC, respectively), made from cellulose acetate hollow fibers (ID 215 ± 15 μm, molecular weight cutoff = 6000; Spectrum Medical Industries, Inc., Los Angeles, CA, USA), were inserted while gently restraining the freely behaving rat. Then, ACSF, which contained (in mM) Na^+ (150), K^+ (3.0), Ca^{2+} (1.2), Mg^{2+} (0.8), Cl^- (155), was perfused at 0.2 μl/min overnight. On the experimental day, the ACSF flow rate was increased to 2 μl/min. After 3–4 h, dialysate samples from both the VTA and NACC were collected at 20-min intervals into vials containing 5 μl 0.1 N HCl and stored at −80 °C until analysis. If VTA GABA was also measured, dialysate samples from the VTA were divided into two portions with one for measurements of DA and the other for determinations of GABA. Frozen samples showed no signs of degradation for up to 1 month in our previous studies.

The treatments for each experiment were as follows. Experiment 1: the rats were injected ip with saline (the same volume as ethanol, $n = 6$) or ethanol at the dose of 1 ($n = 7$) or 2 ($n = 7$) g/kg. Experiment 2: the rats received intra-VTA infusion of 10 μM SB 216641 (a 5-HT_{1B} receptor antagonist [29,52]), 10 μM BRL 15572 (a 5-$HT_{1D/1A}$ receptor antagonist [29,52]), or 10 μM WAY 100635 (a 5-HT_{1A} receptor antagonist [22]). Twenty minutes later, ethanol was administered intraperitoneally at the dose of 1 or 2 g/kg. The numbers of the animal in each treatment group were as follows: $n = 8$ (ethanol 1 g/kg + SB 216641), $n = 7$ (ethanol 1 g/kg + WAY 100635), $n = 7$ (ethanol 1 g/kg + BRL 15572), $n = 8$ (ethanol 2 g/kg + SB 216641), $n = 7$ (ethanol 2 g/kg + WAY 100635), and $n = 6$ (ethanol 2 g/kg + BRL 15572). Experiment 3: the rats received intra-VTA infusion of 10 μM CP 94253 (a 5-HT_{1B} receptor agonist [36]). Twenty minutes later, saline ($n = 6$) or ethanol at the dose of 1 ($n = 6$) or 2 ($n = 7$) g/kg was administered intraperitoneally.

In order to evaluate the implantation of the probe functionally, each dual-probe experiment was finished with infusion of 50 μM of baclofen, a $GABA_B$ receptor agonist, into the VTA probe and the response of extracellular DA in the ipsilateral NACC was determined. A significant decrease (≥50% deduction) in extracellular DA in the ipsilateral NACC after perfusion with baclofen was considered an appropriate implantation of the probe.

2.4. Analytical and histological procedure

For measurement of DA, dialysate samples were injected onto a high performance liquid chromatography (HPLC) system with electrochemical detection. This system consisted of an ESA solvent delivery system (model 580), an ESA microbore column (MD-150 × 1/RP-C18, 3 μM) or an ESA narrowbore column (MD-150 × 2/RP-C18, 3 μM), and an ESA coulochem II electrochemical detector equipped with a dual electrode analytical cell (Model 5041) and a guard cell (Model 5020). The guard cell was set at 400 mV, electrode at 175–200 mV with respect to palladium reference electrodes. A VICI micro-electric two-position valve actuator with a 5-μl (for the microbore column) or 50-μl (for the narrowbore column) injection loop was used for sample injection. The mobile phase contained 75 mM Na_2HPO_4, 1.53 mM sodium dodecyl sulfate, 25 μM EDTA, 100 μl/l triethylamine, 11.5% acetonitrile, and 11.5% methanol (pH 5.6 with H_3PO_4) and was pumped through the system at 0.07 (for the microbore column) or 0.25 (for the narrowbore column) ml/min. Chromatograms were integrated, compared with standards run separately on each experimental day, and analyzed using a computer-based data acquisition system (EZChrom Chromatography Data System, Scientific Software, Inc., San Ramon, CA, USA). The detection limit for dopamine was ~4 fmol at a 2:1 signal-to-noise ratio.

For determination of GABA, an isocratic HPLC system with electrochemical detection was used. This system consisted of an ESA solvent delivery system (model 580), an ESA autosampler (Model 542), a Waters Xterra™ MS column (50 × 3 mm, C18, 2.5 μM), and an ESA coulochem III electrochemical detector equipped with an analytical cell (Model 5011) and a guard cell (Model 5020). The guard cell was set at 650 mV, and the analytical cell at 250 mV (E1)

and 550 mV (E2). The mobile phase contained 100 mM Na_2HPO_4, 0.13 mM Na_2 EDTA, and 28% methanol (pH 6.4 with H_3PO_4) and was pumped through the system at 0.5 ml/min. Pre-column derivatization with *o*-phthaldialdehyde (OPA)/2-mercaptoethanol was performed automatically by the autosampler by mixing 15 μl of the working derivatizing reagent with 20 μl of dialysate samples or working standard solutions for 2 min. The detection limit for GABA was ~500 fmol at a 2:1 signal-to-noise ratio.

After completion of the dialysis, the animals were anesthetized with sodium pentobarbital and then intracardially perfused with buffered saline and 10% formalin prior to decapitation. The brains were removed quickly, and 40-μm thick coronal sections were cut on a freezing microtome, stained with neutral red, and analyzed in the light microscope. The heavy staining of gliosis along the guide cannula track permitted reliable location of the deepest point of penetration. A 2-mm-long (in the NACC) or 1-mm-long (in the VTA) dialysis membrane extended below the tip of the guide cannula. The point of the probe tip was then marked on coronal sections from the atlas of Paxinos and Watson [50].

2.5. Data analysis

Changes in dialysate DA and GABA induced by treatments were expressed as percentages of the baseline in each individual rat. The average DA or GABA levels in three samples immediately preceding the treatment was defined as the baseline (100%). The dialysis data were calculated as mean ± SEM and not corrected for the in vitro probe recovery. A two-way analysis of variance (ANOVA) followed by Tukey's tests was applied. All analyses were performed through computer-based software (SigmaStat). The criterion of significance was set at $P < 0.05$.

3. Results

Only data from animals with correct probe placements (at least 80% of the dialysis membrane in the desired areas) and appropriate accumbal DA responses to perfusion of the VTA with baclofen were included in data analyses. Approximately 70% of the animals that had undergone surgery had both probes correctly implanted in the VTA and NACC and met the functional criterion. Fig. 1 shows the placements of the probe tip in both the VTA and the NACC. The actual placements for the VAT and NACC probes are shown in Fig. 2.

3.1. Effects of systemic ethanol on extracellular DA and GABA in the VTA, and DA in the ipsilateral NACC

These experiments were necessary for this laboratory to establish our own effective doses of ethanol. After DA and GABA in the VTA, and DA in the ipsilateral NACC were

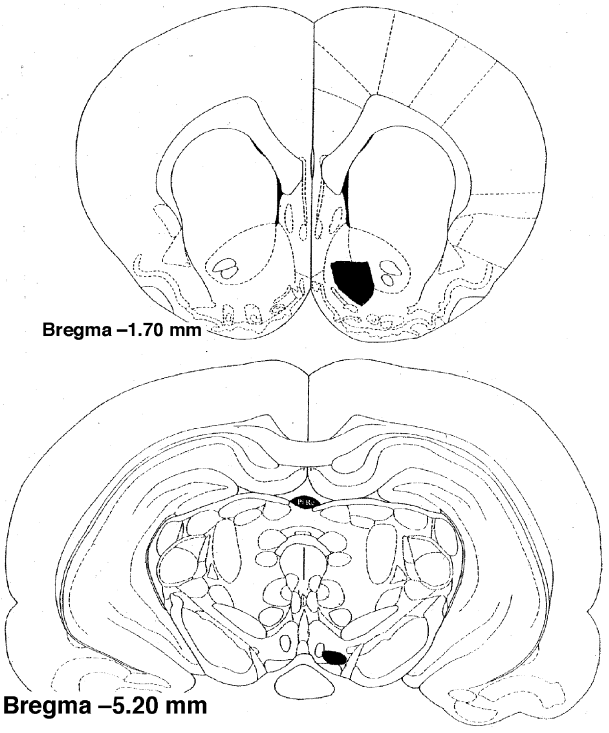

Fig. 1. Diagrammatic representation of microdialysis probe placements in the NACC (the upper panel) and the VTA (the lower panel). The shadowed area represents placements of the probe tips, but not the whole dialysis membrane.

stable, ethanol (1 and 2 g/kg) or saline was injected intraperitoneally and microdialysis was continued for another 2 h. The basal values (fmol/sample) of extracellular DA in the VTA and NACC were as follows: 9.04 ± 1.32 and 29.09 ± 3.09 (the saline group, $n = 6$), 10.99 ± 1.04 and 34.39 ± 2.72 (the 1 g/kg ethanol group, $n = 7$), and 8.94 ± 0.72 and 28.24 ± 2.30 (the 2 g/kg ethanol group, $n = 7$), respectively. There were no statistically significant differences in basal DA in the VTA or the NACC among the ethanol and saline groups. As shown in Fig. 3, intraperitoneal injection of saline had no significant effects on dialysate levels of DA in either the VTA or the ipsilateral NACC. However, administration of ethanol at the doses of 1 and 2 g/kg significantly increased extracellular DA concentrations by ~38% and ~56% of baseline in the VTA ($P < 0.01$ as compared with the saline group, the upper panel of Fig. 3) and ~51% and ~67% of baseline in the NACC ($P < 0.01$ as compared with the saline group, the lower panel of Fig. 3), respectively. As can be seen from this figure, the maximum increases in extracellular DA concentrations in the VTA were temporally correlated with those in the NACC after administration of ethanol at both doses.

Basal GABA levels (fmol/sample) in the VTA dialysate were as follows: 1729.95 ± 141.63 (the saline group, $n = 6$), 1791.73 ± 107.41 (the 1 g/kg ethanol group, $n = 7$), and 1647.43 ± 97.81 (the 2 g/kg ethanol group, $n = 7$). There were no statistically significant differences in the basal GABA levels among the ethanol and saline groups.

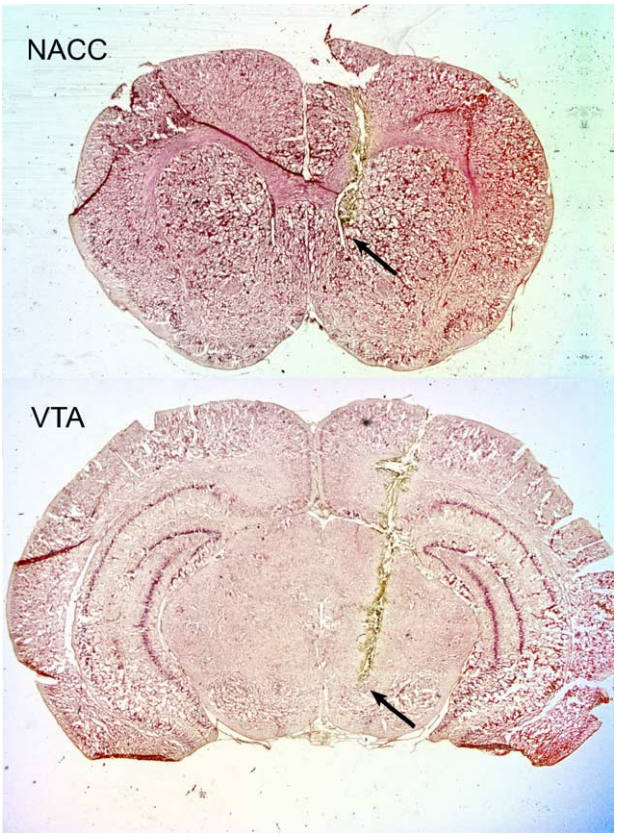

Fig. 2. Representative histological sections stained with neutral red which show tissue damage produced by a guide cannula positioned immediately dorsal to the NACC (the upper panel) or the VTA (the lower panel). A 2-mm-long (in the NACC) or 1-mm-long (in the VTA) dialysis membrane extends below the tip (as indicated by the arrow) of the guide cannula.

Administration of ethanol at the doses of 1 or 2 g/kg did not cause any significant changes in dialysate GABA levels as compared with the saline group (data not shown), suggesting that acute ethanol may not affect extracellular GABA in the VTA under the present experimental conditions. Since no measurable changes in VTA dialysate GABA were detected after administration of ethanol at the doses of 1 or 2 g/kg, GABA contents in the VTA dialysates were no longer assayed in the following experiments.

3.2. Effects of infusion of 5-HT-1 receptor antagonists into the VTA on ethanol-induced DA release in this region and in the ipsilateral NACC (Figs. 4 and 5)

Dual-probe microdialysis was the same as above except that a 5-HT-1 receptor antagonist was infused into the VTA 20 min before ethanol administration and remained throughout the experiments. The following antagonists were used: SB 216641, a 5-HT_{1B} receptor antagonist [29,52], BRL 15572, a 5-$HT_{1D/1A}$ receptor antagonist [29,52], and WAY 100635, and a 5-HT_{1A} receptor antagonist [22]. All these antagonists were infused into the VTA at the concentration of 10 μM in ACSF. The concentration of the antagonists was chosen based on the reports in the literature and ~ 5% of the efficiency of the probe used. It has been shown that 10–100 nM of WAY 100635 potently antagonized the 5-HT_{1A} receptor-mediated effects in the isolated guinea pig ileum [22]. Our previous results showed that local infusion of 10 μM SB 216641 significantly attenuated not only the effects of intra-tegmental CP 93129 on VTA DA and NACC DA but also on VTA GABA [67], suggesting that 10 μM of SB 216641 was sufficient to block VTA 5-HT_{1B} receptors. It has also been reported that the BRL 15572 at the concentration of 0.5 μM blocked the 5-HT_{1D} receptor-mediated effects on in vitro 5-HT release from the rat dorsal raphe nucleus slices [31]. Moreover, infusion of SB 216641, WAY 100635, or BRL 15572 at the concentration of 10 μM into the VTA for 2 h did not significantly alter extracellular DA levels in either the VTA or the ipsilateral NACC [67].

Figs. 4 and 5 show comparisons of ethanol (1 and 2 g/kg)-induced DA release in the VTA and the NACC in the presence and absence of SB 216641, WAY 100635, or BRL 15572. As shown in these figures, ethanol-induced DA releases, except those occurred in the VTA after 1 g/kg

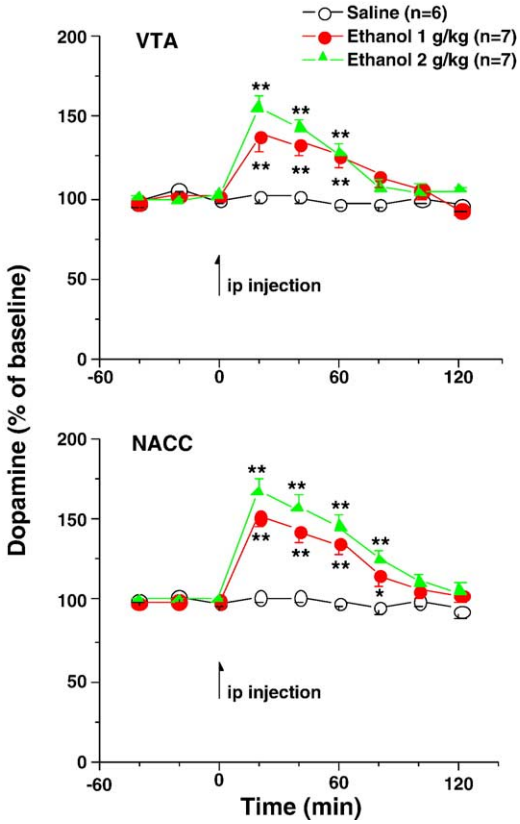

Fig. 3. Effects of acute ethanol on extracellular DA in the VTA (the upper panel) and the ipsilateral NACC (the lower panel). Microdialysis probes placed in the VTA and the NACC were perfused with ACSF simultaneously. Saline or ethanol (1 and 2 g/kg) was administered by intraperitoneal injection indicated by the arrow. Results are mean ± SEM from six to seven animals. *$P < 0.05$, **$P < 0.01$ as compared with the saline group (two-way ANOVA followed by Tukey's tests).

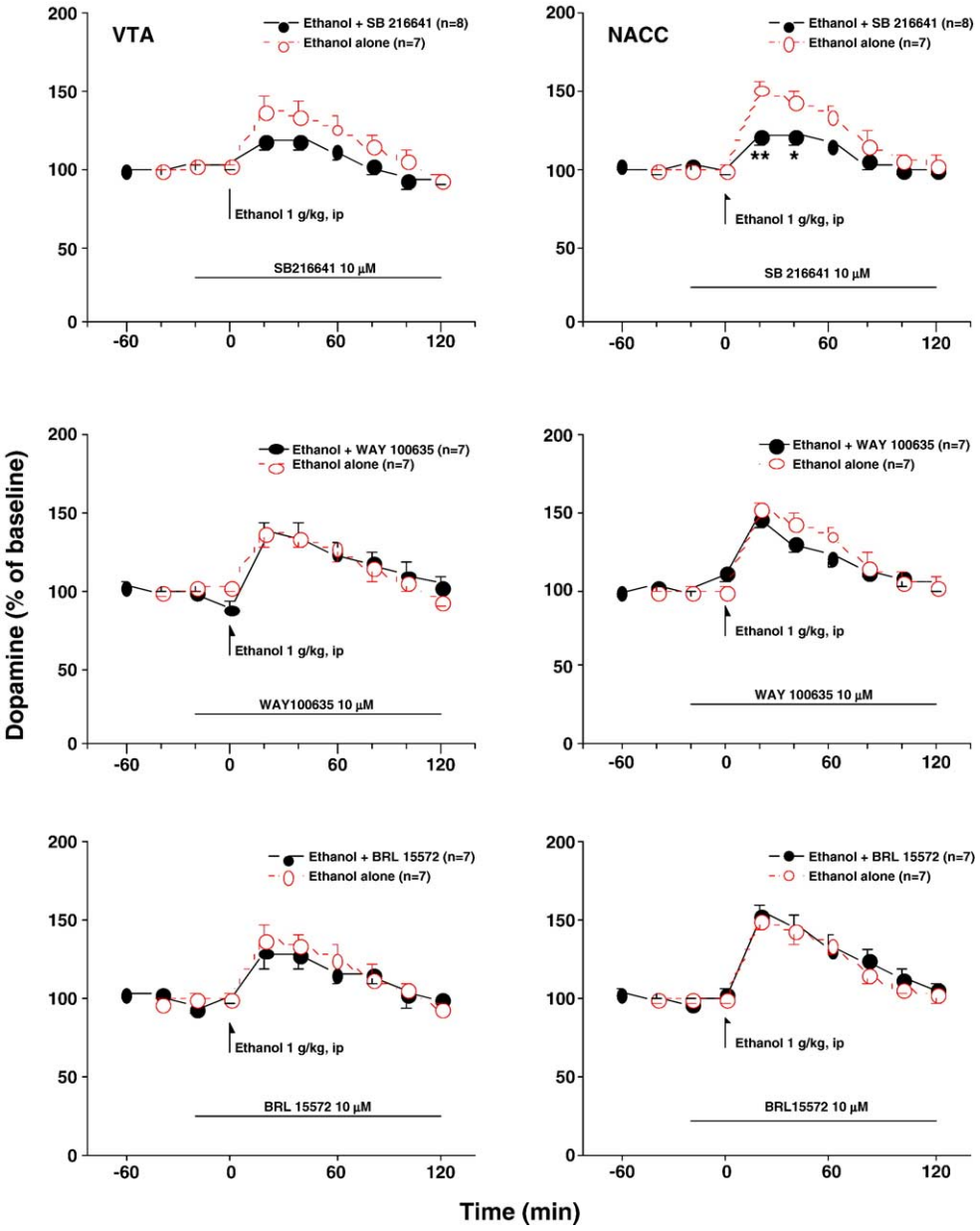

Fig. 4. Comparisons of ethanol (1 g/kg)-induced DA release in the VTA (the left panel) and the ipsilateral NACC (the right panel) in the presence and absence of SB 216641 (upper), WAY 100635 (middle), or BRL 15572 (lower). SB 216641 (10 μm), WAY 100635 (10 μm), or BRL 15572 (10 μm) was infused into the VTA 20 min before ethanol (1 g/kg ip) administration and remained throughout the experiments. Results are mean ± SEM from seven to eight animals. The data of the ethanol alone group were obtained from Fig. 3. The basal DA levels (fmol/sample) in the VTA and the NACC were as follows: 10.99 ± 1.04 and 34.39 ± 2.72 (the ethanol alone group, $n = 7$), 11.16 ± 0.81 and 46.78 ± 4.84 (the ethanol + SB 216641 group, $n = 8$), 10.31 ± 1.18 and 36.92 ± 3.96 (the ethanol + WAY 100635 group, $n = 7$), and 12.32 ± 1.37 and 37.71 ± 3.47 (the ethanol + BRL 15572 group, $n = 7$), respectively. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with the ethanol alone group (two-way ANOVA followed by Tukey's tests).

of ethanol (the upper left of Fig. 4), were all significantly attenuated by co-administration of SB 216641. In the presence of SB 216641, ethanol (1 and 2 g/kg)-induced NACC DA release (the upper right of Figs. 4 and 5) and ethanol (2 g/kg)-induced VTA DA release (the upper left of Fig. 5) were all significantly lower than those in the absence of SB 216641. However, co-administration of WAY 100635 or BRL 15572 had no significant effects on ethanol-induced DA release in either the VTA or the NACC.

3.3. Effects of infusion of CP 94253 into the VTA on ethanol (1 and 2 g/kg)-induced DA release in the ipsilateral NACC (Figs. 6 and 7)

These experiments were designed to investigate further the involvement of VTA 5-HT_{1B} receptors in modulation of the effects of ethanol on DA release in the NACC. Dual-probe microdialysis was the same as described in Section 3.1 except that CP 94253 (10 μM, a 5-HT_{1B} receptor agonist [36]) was infused into the VTA 20 min

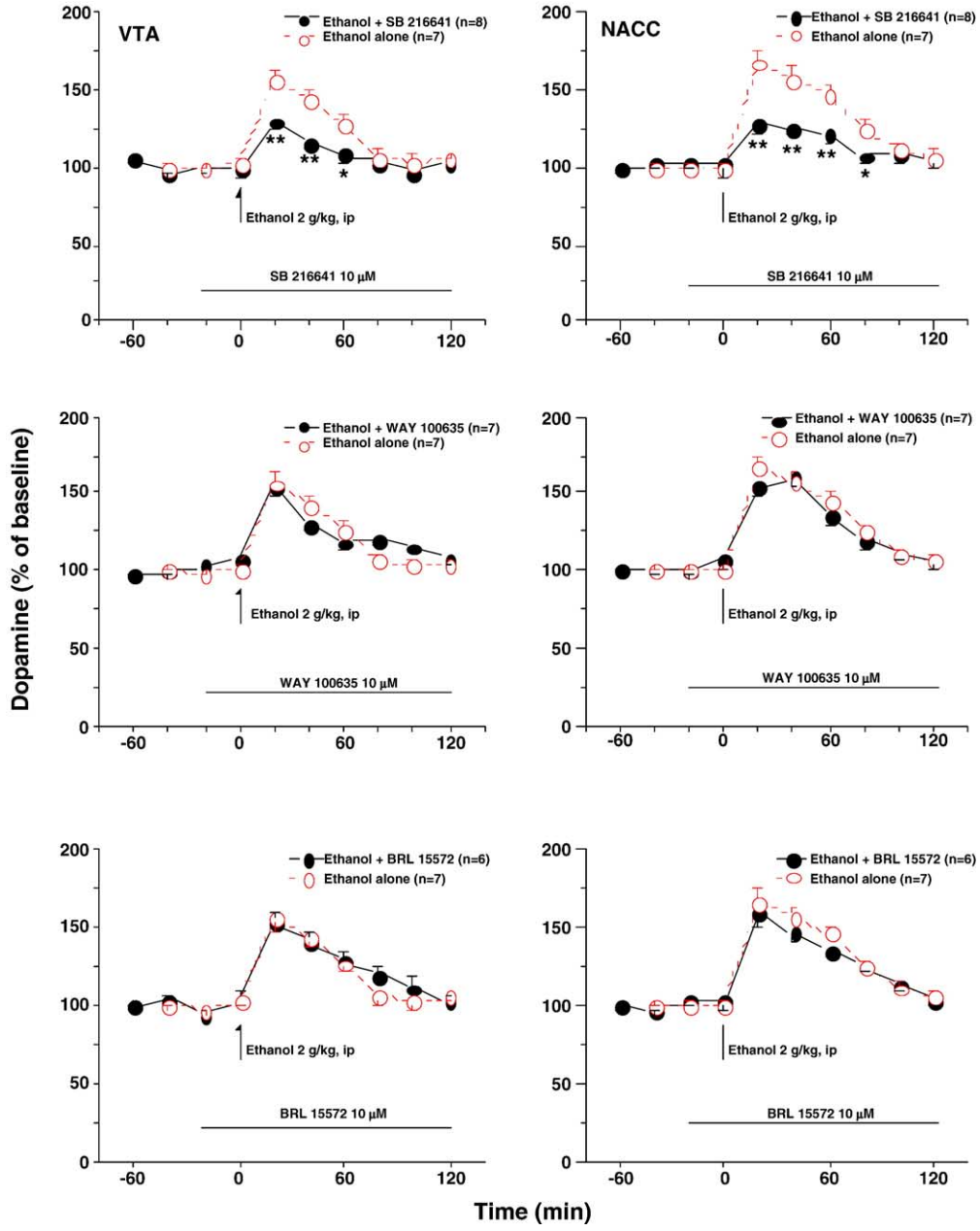

Fig. 5. Comparisons of ethanol (2 g/kg)-induced DA release in the VTA (the left panel) and the ipsilateral NACC (the right panel) in the presence and absence of SB 216641 (upper), WAY 100635 (middle), or BRL 15572 (lower). SB 216641 (10 μm), WAY 100635 (10 μm), or BRL 15572 (10 μm) was infused into the VTA 20 min before ethanol (2 g/kg ip) administration and remained throughout the experiments. Results are mean ± SEM from six to eight animals. The data of the ethanol alone group were obtained from Fig. 3. The basal DA levels (fmol/sample) in the VTA and the NACC were as follows: 8.94 ± 0.72 and 28.24 ± 2.30 (the ethanol alone group, $n = 7$), 8.24 ± 0.83 and 26.83 ± 2.53 (the ethanol ± SB 216641 group, $n = 8$), 9.25 ± 1.04 and 28.79 ± 2.20 (the ethanol + WAY 100635 group, $n = 7$), and 9.06 ± 0.93 and 27.64 ± 1.85 (the ethanol + BRL 15572 group, $n = 6$), respectively. *$P < 0.05$, **$P < 0.01$ as compared with the ethanol alone group (two-way ANOVA followed by Tukey's tests).

before ethanol injection and remained throughout the experiments. In a separate group of rats, perfusion of the VTA with CP 94253 (10 μM in ACSF) followed by the saline injection did not significantly alter extracellular DA levels in the ipsilateral NACC (Figs. 6 and 7). As shown in Figs. 6 and 7, however, administration of CP 94253 significantly changed the time course of extracellular DA concentrations following ethanol administration. In the absence of CP 94253, extracellular DA in the NACC increased rapidly to the maximum level after administration of ethanol at the doses of 1 (Fig. 6) and 2 g/kg (Fig. 7), then declined and reached the control level at 80 min after ethanol injection. However, in the presence of CP 94253, extracellular DA still remained significantly high levels as compared with either the saline or the ethanol alone group at 80–120 min after administration of ethanol at the both doses (Figs. 6 and 7). The results indicated that the treatment with CP 94253 significantly prolonged the

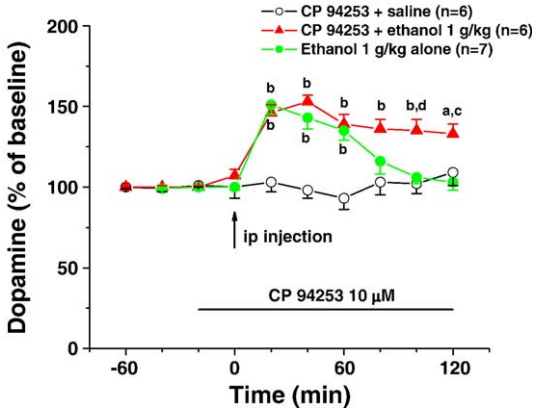

Fig. 6. Effects of co-administration of CP 94253 on ethanol (1 g/kg)-induced NACC DA release. CP 94253 (10 μM) was infused into the VTA through a probe as indicated by the bar. Ethanol (1 g/kg) or saline was injected intraperitoneally as indicated by the arrow. Extracellular DA in the ipsilateral NACC was measured by a second probe in this region. The data of the ethanol alone group was obtained from Fig. 3. $^{a}P < 0.05$, $^{b}P < 0.01$ as compared with the CP 94253 + saline group; $^{c}P < 0.05$, $^{d}P < 0.01$ as compared with the ethanol alone group (two-way ANOVA followed by Tukey's tests). The basal DA levels (fmol/sample) were as follows: 34.39 ± 2.72 (the ethanol alone group), 37.02 ± 3.64 (the CP 94253 + saline group), and 35.97 ± 3.73 (the CP 94253 + ethanol group).

effects of ethanol (1 and 2 g/kg) on the extracellular DA in the NACC although the maximum increases of NACC DA after co-administration of CP 94253 and ethanol did not significantly differ from those after administration of ethanol alone.

4. Discussion

Previous studies show that, in addition to classical DA release from synapses in their terminal areas, VTA DA neurons release DA from their somata and dendrites [1,11]. Accumulating evidence suggests that the release of DA in the VTA is regulated by mechanisms similar to those of axonal release although the former appears to be less sensitive to pharmacological manipulations. Thus, DA release in the VTA, similar to extracellular DA in the NACC, was augmented following potassium [35] or veratridine depolarization [33], and reduced after tetrodotoxin or calcium omission [10,12], suggesting that a depolarization-induced, exocytosis-mediated, somatodendritic release of DA occurred in the VTA under basal conditions. As a result, somatodendritic DA in the VTA, like that released from axon terminals in the NACC, can also be used as an index of the activity of mesolimbic DA neurons.

The present data show that systemic administration of ethanol at the doses of 1 and 2 g/kg increases extracellular DA concentrations not only in the NACC but also in the VTA in the same animal. Kohl et al. [37] also reported that systemic administration of ethanol at the doses of 2 and 3 g/kg simultaneously increased DA concentrations in the VTA and the ipsilateral NACC when assessed with dual-probe brain dialysis. Simultaneous elevations in the extracellular levels of DA in both cell body and terminal areas suggest that systemic administration of ethanol increases the firing rate of VTA DA neurons. Our results are in agreement with in vivo [25] and in vitro [7,8] electrophysiological experiments, and with previous microdialysis studies, which indicated that systemic administration of ethanol could increase the release of DA in the NACC [26,63] and the VTA [10] in separate animals. Interestingly, administration of nicotine, a drug that increases mesolimbic DA transmission by similar mechanisms as ethanol [45,47], also increased both somatodendritic DA release in the VTA and synaptic DA release in the NACC [68].

It has been reported that the excitatory action of ethanol on VTA DA neurons can be potentiated by 5-HT and 5-HT reuptake blockers [6,58]. This 5-HT-induced potentiation may be related to the stimulation of various 5-HT receptors. One of the 5-HT receptors suggested to have a modulatory effect on VTA DA neurons is the 5-HT_{1B} subtype. The main purpose of this study was to test the hypothesis that VTA 5-HT_{1B} receptors may modulate ethanol-induced increases in mesolimbic DA neurotransmission. This hypothesis was based on the observations that VTA 5-HT_{1B} receptors were involved in regulation of mesolimbic DA neurotransmission [64,67] and that local administration of ethanol into the VTA via a dialysis probe increased extracellular concentrations of 5-HT in this region [65], which could result in increased activation of VTA 5-HT_{1B} receptors.

To assess the involvement of 5-HT_{1B} receptors in ethanol's actions, WAY-100635, SB 216641, and BRL 15572 were used. The affinities (given as pK_i) of these

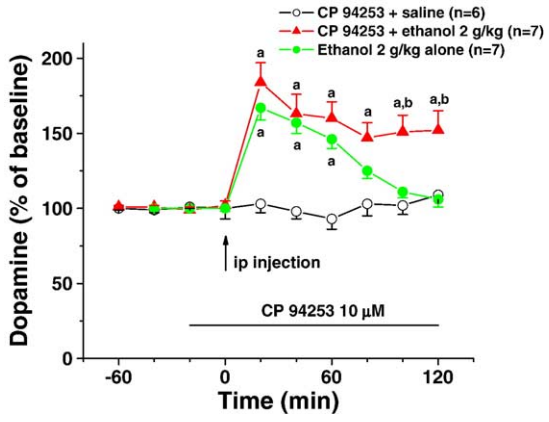

Fig. 7. Effects of co-administration of CP 94253 on ethanol (2 g/kg)-induced NACC DA release. CP 94253 (10 μM) was infused into the VTA through a probe as indicated by the bar. Ethanol (2 g/kg) or saline was injected intraperitoneally as indicated by the arrow. Extracellular DA in the ipsilateral NACC was measured by a second probe in this region. The data of the ethanol alone group were obtained from Fig. 3. The data of the CP 94253 + saline group were obtained from Fig. 6. $^{a}P < 0.01$ as compared with the CP 94253 + saline group; $^{b}P < 0.01$ as compared with the ethanol alone group (two-way ANOVA followed by Tukey's tests). The basal DA levels (fmol/sample) were as follows: 28.24 ± 2.30 (the ethanol alone group), 37.02 ± 3.64 (the CP 94253 + saline group), and 34.99 ± 3.46 (the CP 94253 + ethanol group).

antagonists for 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptor subtypes are as follows: WAY 100635: 8.9, <6, and <6; SB 216641: 6.3, 9.0, and 7.6; BRL 15572: 7.7, 6.1, and 7.9 [29,52]. All these drugs were tested for their impacts on the actions of systemic ethanol on DA release in both the VTA and the NACC. The reason for which WAY 100635 was used is as follows. First, although SB 216641 is "selective" for the 5-HT_{1B} receptor, it may have weak antagonism at 5-HT_{1A} receptors when 10 μM is used. Moreover, the expression of 5-HT_{1A} receptors in the VTA has been demonstrated by autoradiographic [51] and in situ hybridization studies [62], providing anatomical evidence for potential interactions between 5-HT_{1A} receptors and VTA DA neurons. Indeed, it has been reported that administration of a 5-HT_{1A} receptor agonist has a biphasic effect on mesolimbic DA neurons with low doses being stimulatory and high doses being inhibitory on the neuronal firing rate [40,41]. As a result, the potential involvement of 5-HT_{1A} receptors in ethanol's actions needs to be addressed. If 5-HT_{1A} receptors are involved in the actions of ethanol, WAY 100635 (pK_i = 8.9 for the 5-HT_{1A} receptor), and BRL 15572 (pK_i = 7.7 for the 5-HT_{1A} receptor) would to some degree antagonize the effects of ethanol on VTA DA or NACC DA. However, this is not the case. The data presented here show that administration of neither WAY 100635 nor BRL 15572 into the VTA antagonizes the effects of systemic ethanol on extracellular DA in either the VTA or the NACC. These results are in opposition with the involvement of VTA 5-HT_{1A} receptors in ethanol's effects.

SB 216641 has been reported to have high affinity and selectivity for 5-HT_{1B} over 5-HT_{1D} receptors [46]. This drug shows more than 10-fold higher selectivity at 5-HT_{1B} (pK_i = 9.0) compared to 5-HT_{1D} (pK_i = 7.6) receptors [52]. BRL 15572 is recently identified as a selective 5-HT_{1D} receptor antagonist. It is more than 60-fold selective for 5-HT_{1D} over 5-HT_{1B} receptors [52]. It has been reported that CP 93129-induced inhibition of 5-HT release was antagonized by SB 216641 but remained unaffected in the presence of BRL 15572 [31]. The present data show that systemic ethanol-induced augmentations of VTA DA and NACC DA were significantly attenuated by local administration of SB 216641 but not by BRL 15572. These data are consistent with the involvement of VTA 5-HT_{1B} receptors, but not VTA 5-HT_{1D} receptors, in mediating the ethanol-induced excitation of VTA DA neurons.

In order to further investigate the involvement of VTA 5-HT_{1B} receptors in ethanol-induced activation of mesolimbic DA neurons, the 5-HT_{1B} receptor agonist CP 94253 was used. CP 94253 has at least a 40-fold and a 20-fold greater selectivities for the 5-HT_{1B} receptor over the 5-HT_{1A} and the 5-HT_{1D} receptor, respectively (K_i values are 89, 2, 860, 49, and 1600 nM for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_2 receptors, respectively) [36]. The data presented here showed that local administration of 10 μM CP 94253 into the VTA did not significantly alter extracellular DA concentrations in the ipsilateral NACC. This result suggests that this concentration of CP 94253 may not cause sufficient activation of VTA 5-HT_{1B} receptors to produce significant increases in the activity of mesolimbic DA neurons under the present experimental conditions. However, this concentration of CP 94253 did enhance the effects of ethanol on extracellular DA in the NACC. The present data show that the treatment with CP 94253 significantly prolongs the effects of ethanol although it does not significantly enhance ethanol's peak effects. As shown in Figs. 6 and 7, extracellular DA remains significantly high at 80–120 min after ethanol in the presence of CP 94253 as compared with that in the absence of CP 94253. It was reported that the blood alcohol concentration (BAC) rose progressively to a peak at 20–40 min and then decreased thereafter, which was temporally correlated with changes of extracellular DA and 5-HT [63,69], following intraperitoneal injection of 1 or 2 g/kg of ethanol [19,42]. Therefore, our observation suggests that enhancement of ethanol's effects by CP 94253 occurs mainly during the descending limb of the BAC. Although the precise reasons for this type of enhancement are not presently understood, it is conceivable that this phenomenon may be due to additive or synergetic activation of VTA 5-HT_{1B} receptors by CP 94253 and released 5-HT by ethanol. The observed lack of further increases of ethanol's peak effects by CP 94253 may be a result of the VTA 5-HT_{1B} receptors already being maximally (or near maximally) activated by ethanol-evoked 5-HT, consequently, leading to little potential for further activation by CP 94253. Together with the data obtained with the 5-HT_{1B} receptor antagonist, the results support the suggestion that activation of 5-HT_{1B} receptors within the VTA may contribute to systemic ethanol-induced increases of mesolimbic DA neuronal activities.

The present results also show that although SB 216641 significantly attenuated ethanol-induced increases in DA release in both the VTA and NACC it did not completely block ethanol's effects. For example, injection of ethanol at the doses of 1 and 2 g/kg still caused NACC DA to increase to 121% (the upper right of Fig. 4) and 127% (the upper right of Fig. 5) of baseline, respectively, in the presence of SB 216641. This result may imply that, in addition to 5-HT_{1B} receptors, other receptors and/or mechanisms may also be involved in ethanol's neurochemical effects. Previous reports showed that local administration of a 5-HT_3 receptor antagonist antagonized systemic ethanol-induced increases of DA release in the VTA [10] or the NACC [9]. Moreover, the studies by Ericson et al. [18] and Blomqvist et al. [3] suggest that ethanol may elevate NACC DA via activation of VTA nicotinic acetylcholine receptors because ethanol-induced DA release in the NACC was antagonized by intra-tegmental administration of the acetylcholine receptor antagonist mecamylamine. Taken together, the results suggest that, in addition to 5-HT_{1B} receptors, multiple neurotransmitter receptors within the VTA may be involved in mediating the ethanol-evoked stimulation of mesolimbic DA neurons.

The mechanisms by which VTA 5-HT_{1B} receptors are involved in mediating ethanol's effects on mesolimbic DA transmission are unknown. It has been found that VTA DA neurons are under GABAergic inhibitory control [2]. Moreover, previous studies showed that activation of 5-HT_{1B} receptors inhibited high potassium-evoked [^{3}H] GABA release from rat VTA slices [66] and that GABA neurotransmission within the VTA may contribute to the 5-HT_{1B} receptor-mediated disinhibition of mesolimbic DA neurons [67]. These findings prompted us to speculate that the 5-HT_{1B} receptor-mediated inhibition of VTA GABA transmission may contribute to the stimulatory effect of ethanol on VTA DA neurons. Therefore, potential effects of systemic ethanol on VTA GABA were examined in this study. Unfortunately, the data presented here indicated that administration of ethanol at the dose of 1 or 2 g/kg did not significantly alter extracellular GABA in the VTA but did increased DA concentrations in both the VTA and the NACC. These results do not support our speculation. Cowen et al. [14] also reported that administration of ethanol (1 g) by gavage had no significant effects on extracellular GABA in the VTA or the substantia nigra measured by microdialysis. However, it should be pointed out that the technique of in vivo microdialysis requires long sampling time due to the low flow rates employed, and as such can only readily quantify substantial changes in release of neurotransmitters that maintained for a period of time. It is possible that administration of ethanol evokes a transient change in VTA GABA levels that is masked in a 20-min sample. In addition, previous microdialysis studies indicated that the part of GABA as measured by microdialysis did not fulfill the classical criteria for neuronal release [56], suggesting that GABA levels monitored by microdialysis probes may derive from non-neuronal pools in addition to the neuronal origin. Consequently, it is also possible that potential alterations in neuronal GABA release resulting from ethanol administration may only cause a small change in total extracellular GABA levels that cannot be detected by our HPLC system. As a result, an inability to detect measurable changes in the release of endogenous GABA in the VTA following ethanol does not necessarily mean that VTA GABA is not a target of acute ethanol. Further studies using more sensitive techniques to detect ethanol's effects on GABA in the VTA may be needed. In addition to regulating GABA release, 5-HT_{1B} heteroreceptors also provide an inhibitory modulation of glutamate release [46]. Glutamatergic afferents to the VTA are thought to play a role in regulating the activity of DA neurons in this region [5,34], and increased glutamatergic tone in the VTA has been reported to produce both excitatory and inhibitory effects on DA cell activity [21,55]. Thus, studies are currently underway in this laboratory to explore the possibility that 5-HT_{1B} receptor-mediated alterations in VTA glutamate efflux may be involved in part in the stimulatory effects of ethanol on VTA DA neurons.

The present study provides evidence that suggests that the VTA is one locus in the brain where 5-HT_{1B} receptors can modulate the neurochemical effects produced by peripherally administered ethanol. Since increased mesolimbic DA neurotransmission has been implicated in ethanol's rewarding effects [38], the present findings showing the involvement of VTA 5-HT_{1B} receptors in mediating ethanol's neurochemical effects may explain in part why 5-HT_{1B} receptors play a role in modifying the reinforcing [15,43,53], intoxicating [15], and discriminative stimulus effects of ethanol [23,28] as well as regulating its voluntary intake [15,43,44]. For example, the reported suppression of alcohol intakes by 5-HT_{1B} receptor agonists [30,57,60] may be caused, at least in part, by the drug-induced potentiation of ethanol's actions on mesolimbic DA neurotransmission. Interestingly, a recent study by O'Dell and Parsons [48] showed that VTA 5-HT_{1B} receptors also modulated cocaine-induced increases in NACC DA levels. This modulation of cocaine's neurochemical effects by 5-HT_{1B} receptors are consistent with the behavioral studies showing that 5-HT_{1B} receptors play a role in the regulation of cocaine's reinforcing and discriminative stimulus effects [20,49].

In summary, the present results indicate that activation and blockade of VTA 5-HT_{1B} receptors potentiates and attenuates the ethanol-induced increases in extracellular DA concentrations in both the VTA and the ipsilateral NACC, respectively. The data support the suggestion that VTA 5-HT_{1B} receptors may be involved in part in mediating the activating effects of ethanol on mesolimbic DA neurons.

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