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13. ABSTRACT (Maximum 200 Words)

The experiments under Specific Aim 1 have been completed. Dual-probe microdialysis was performed as before. Ethanol (1 and 2 g/kg) or saline was injected ip to animals and extracellular dopamine (DA) and GABA in the ventral tegmental area (VTA), and DA in the ipsilateral nucleus accumbens (NACC) were measured. Then, SB 216641 (10 μM, a 5-HT1B receptor antagonist), WAY 100635 (10 μM, a 5-HT1A receptor antagonist), BRL (10 μM, a 5-HT1D/1A receptor antagonist), or CP 94253 (10 μM, a 5-HT1B receptor agonist) was infused into the VTA 20 min before ethanol administration (1 and 2 g/kg) until the end of the experiments. The results showed that administration of ethanol at the doses of 1 and 2 g/kg increased extracellular DA concentrations in both areas. The time course of extracellular DA in the VTA after ethanol is similar to that in the NACC. Administration of ethanol at the same doses did not produce significant changes in extracellular VTA GABA. Co-administration of SB 216641, but not WAY 100635 or BRL 15572, attenuated ethanol-evoked DA release in both areas. Co-administration of CP 94253 prolonged the effects of ethanol on extracellular NACC DA. The results are consistent with the hypothesis that activation and blockade of VTA 5-HT-1B receptors potentiates and attenuates ethanol's effects on DA transmission in the ipsilateral NACC, respectively. These observations may in part explain why 5-HT-1B receptors play a role in ethanol's behavioral effects. The experiments under Specific Aim 2 were also initiated in 5-HT1B receptor knockout (KO) and their counterparts wild-type (WT) mice. The results showed that the 5-HT-1B/1A receptor agonist RU 24969 increased extracellular NACC DA concentrations in the WT but not in the KO mice. The results provide additional support to the hypothesis that 5-HT-1B receptors are involved in modulation of mesolimbic dopaminergic neurotransmission.

14. SUBJECT TERMS
Ethanol, Dopamine, Serotonin, 5-HT1B receptor, GABA, Nucleus accumbens, Ventral tegmental area, 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 the first hypothesis of Specific Aim 1 were completed in Year 2. The data reported in the second annual report have already been published in *Brain Research* (1021: 82-91, 2004, please see the appendices). The experiments related to the second hypothesis were continued and have been completed during the third year of the project. The following data were obtained in Year 3 and have not been reported in the previous reports.

1. Effects of acute ethanol on extracellular DA and GABA in the VTA and DA in the ipsilateral NACC. In these experiments, dual-probe microdialysis was used in adult male Sprague-Dawley rats as described previously[1]. One probe was inserted into the VTA and the other in the ipsilateral NACC. Both probes were perfused with artificial cerebrospinal fluid (ACSF) at the rate of 1.5 – 2.0 µl/min. The dialysates from the VTA and NACC were collected at 20 min of intervals, and assayed via the HPLC-EC (high performance liquid chromatography coupled with electrochemical detection) system for DA in the NACC, and GABA and DA in the VTA (The VTA dialysates were divided into two portions: one for GABA assay and the other for DA measurement). After the release of transmitters in the VTA and NACC was stable, ethanol (1 and 2 g/kg) or saline was injected ip and microdialysis was continued for another 2 h. These experiments were necessary for this laboratory to establish our own effective doses of ethanol.

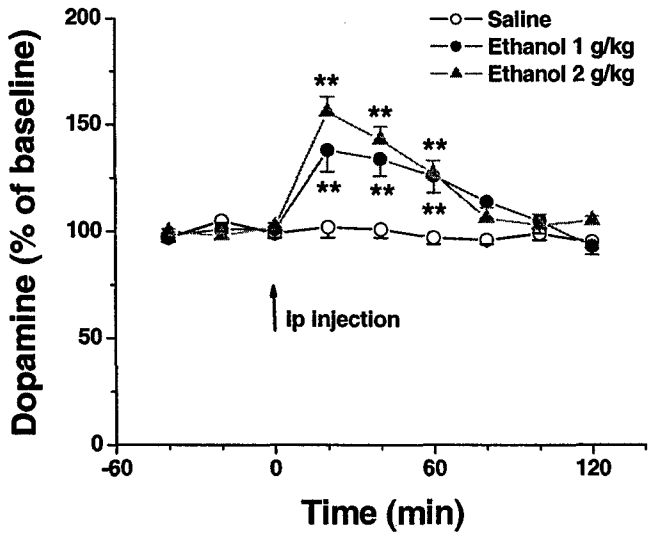

Fig 1. Effects of acute ethanol on extracellular DA in the VTA. Microdialysis probes placed in both the VTA and ipsilateral NACC were perfused simultaneously. Saline or ethanol (1 and 2 g/kg) was administered by ip injection indicated by the arrow. Results are mean ± SEM from six to seven animals. **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

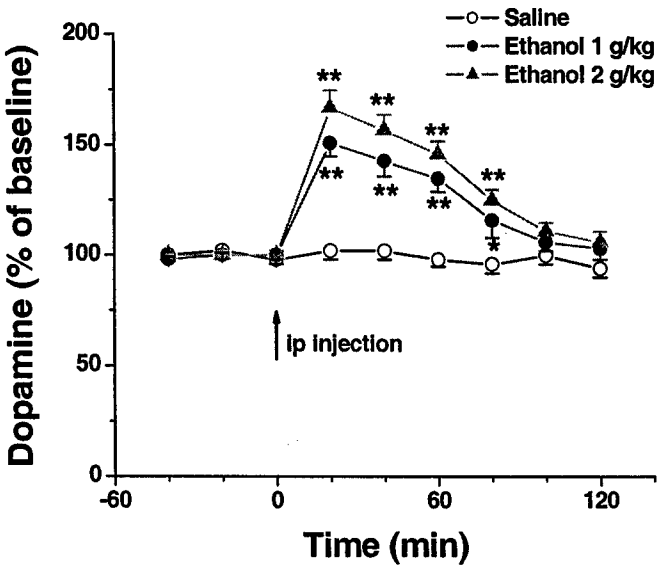

Fig 2. Effects of acute ethanol on extracellular DA in the NACC. Microdialysis probes placed in both the VTA and ipsilateral NACC were perfused simultaneously. Saline or ethanol (1 and 2 g/kg) was administered by ip injection indicated by the arrow. Results are mean ± SEM from six to seven animals. *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

The basal values (fmol/sample) of extracellular DA in the VTA and NACC were: 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 Figs 1 and 2, intraperitoneal injection of saline had no significant effects on dialysate levels of DA from either the VTA or the ipsilateral NACC. However, administration of ethanol at the doses of 1 and 2 g/kg increased extracellular DA concentrations by ~38% and ~56% of baseline in the VTA, and ~51% and ~ 67% of baseline in the NACC, respectively. The maximum increases in DA release in the VTA were temporally correlated with those in the NACC after administration of ethanol at both doses. These data are consistent with the previous studies carried out in this[2] and other[3,4] laboratories showing that acute ethanol increased terminal or somatodendritic DA release in separate animals. Moreover, the present experiments, by taking advantages of simultaneous measurements of DA release in both the VTA and NACC in the same animals, extend these findings to show that ethanol-induced increases in somatodendritic and axon terminal DA release are correlative temporally and similar in magnitude. Simultaneous demonstration of both somatodendritic and axon terminal DA release in the same animals may provide more complete profiles regarding the effects of ethanol on the mesolimbic DA pathway. The observed similarity in the time course of DA between cell body and terminal areas after ethanol is opposite to that observed with cocaine which increases extracellular DA by a different mechanism. The previous study carried out in this laboratory showed that systemic cocaine produced different DA time courses in the VTA and the NACC with much longer increases in the former than in the latter[12].

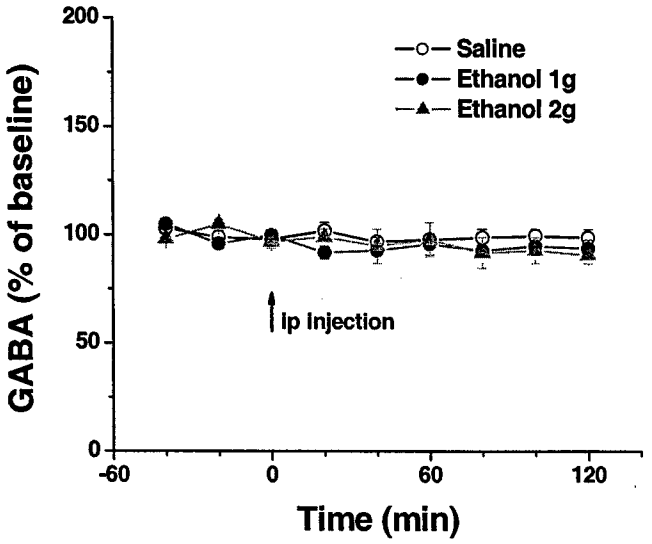

Fig 3. Effects of acute ethanol on extracellular GABA in the VTA. Microdialysis probes placed in both the VTA and ipsilateral NACC were perfused simultaneously. Saline or ethanol (1 and 2 g/kg) was administered by ip injection indicated by the arrow. Results are mean ± SEM from six to seven animals. There were no statistically significant differences between the ethanol and saline groups.

Fig 3 shows the effects of acute ethanol on extracellular GABA in the VTA. Basal extracellular GABA levels (pg/sample) in the VTA were: 178.36 ± 14.60 (the saline group, n = 6), 184.73 ± 11.07 (the 1 g/kg ethanol group, n = 7), and 169.85 ± 10.08 (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. As shown in this figure, 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, suggesting that acute ethanol may not affect extracellular GABA in the VTA under the present experimental conditions. Cowen et al.[5] also reported that administration of ethanol (1 g) by gavage had no significant effects on extracellular GABA in the VTA or the substantia nigra. However, it must be remembered 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 are maintained for a period of time. It is possible that acute ethanol administration evoked a transient change in GABA release in the VTA that was masked in a 20-min sample. In addition, as mentioned in the grant application, part of GABA collected by microdialysis probes may originate from non-neuronal stores[6]. Therefore, it is also possible that potential alterations in exocytotic 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. Regardless of the explanations, 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 different techniques to detect ethanol's effects on the GABA system in the VTA may be needed. Since no measurable changes in VTA dialysate GABA were detected after administration of ethanol at the doses of 1 or 2 g/kg, attention were paid to VTA DA and NACC DA in the following experiments.

2. The effects of infusion of a 5-HT-1B receptor antagonist into the VTA on ethanol-induced DA release in this region and in the ipsilateral NACC. Dual-probe microdialysis was employed the same as above except that a 5-HT-1 receptor antagonist was infused into the VTA 20 minutes before ethanol until the end of the experiments. In these experiments, the following antagonists were used: SB 216641, a 5-HT-1B receptor antagonist[7], BRL 15572, a 5-HT-1D/1A receptor antagonist[7], and WAY 100635, a 5-HT-1A receptor antagonist[8]. The affinities (given as p*Ki*) of these antagonists for related 5-HT-1 receptor subtypes are listed in the following table. All these antagonists were infused into the

VTA at the concentration of 10 μM in ACSF. The previous results showed that infusion of SB 216641, but not BRL 15572 or WAY 100635, at this concentration antagonized not only the effects of intra-tegmental CP 93129 on VTA DA and NACC DA but also on VTA GABA[1], suggesting that 10 μM of SB 216641 was sufficient to block VTA 5-HT-1B receptors. The previous studies also showed that 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[1].

Compound	Receptor subtype (pK*i*)		
	5-HT-1A	5-HT-1B	5-HT-1D
SB-216641	6.3	9.0	7.6
BRL-15572	7.7	6.1	7.9
WAY-100635	8.9	< 6	< 6

Figs 4-7 show comparisons of ethanol (1 and 2 g/kg)-induced DA release in the VTA and NACC in the presence and absence of SB 216641, WAY 100635, or BRL 15572, respectively. As shown in these figures, ethanol-induced DA releases, except those occurred in the VTA after 1 g/kg of ethanol (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 (Figs 6-7) and ethanol (2 g/kg)-induced VTA DA release (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. The present results show that activation of VTA 5-HT-1B receptors may contribute to ethanol-stimulated somatodendritic and axon terminal DA release, suggesting the involvement of VTA 5-HT-1B receptors in ethanol-induced activation of mesolimbic DA neurons.

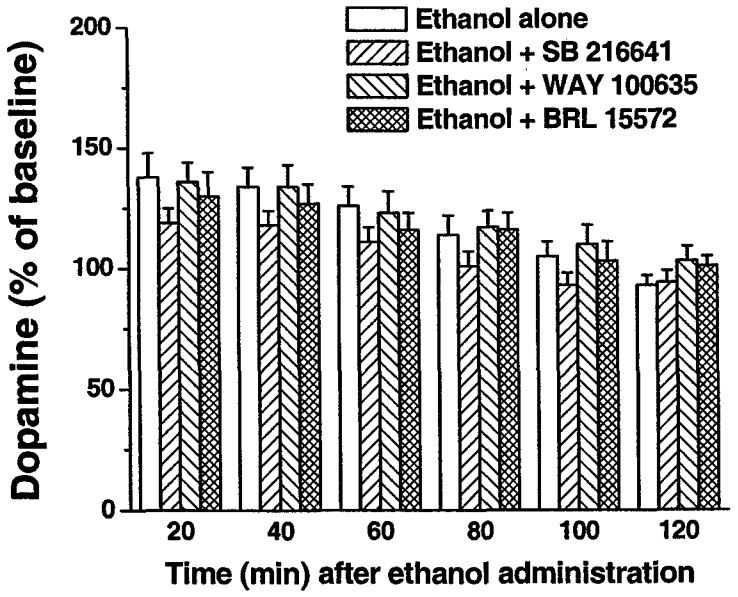

Fig 4. Comparisons of ethanol (1 g/kg)-induced VTA DA release in the presence and absence of SB 216641, WAY 100635, or BRL 15572. SB 216641 (10 μm), WAY 100635 (10 μm), or BRL 15572 (10 μm) was infused into the VTA 20 min before ethanol ip injection until the end of the experiments. Results are mean ± SEM from seven to eight animals. The data of the ethanol alone group were obtained from Fig 1. There were no significant differences between the ethanol alone group and other groups (two-way ANOVA followed by Tukey's tests). The basal DA levels (fmol/sample) in the VTA were: 10.99 ± 1.04 (the ethanol alone group, n=7), 11.16 ± 0.81 (the ethanol + SB 216641 group, n=8), 10.31 ± 1.18 (the ethanol + WAY 100635 group, n=7), and 12.32 ± 1.37 (the ethanol + BRL 15572 group, n=7).

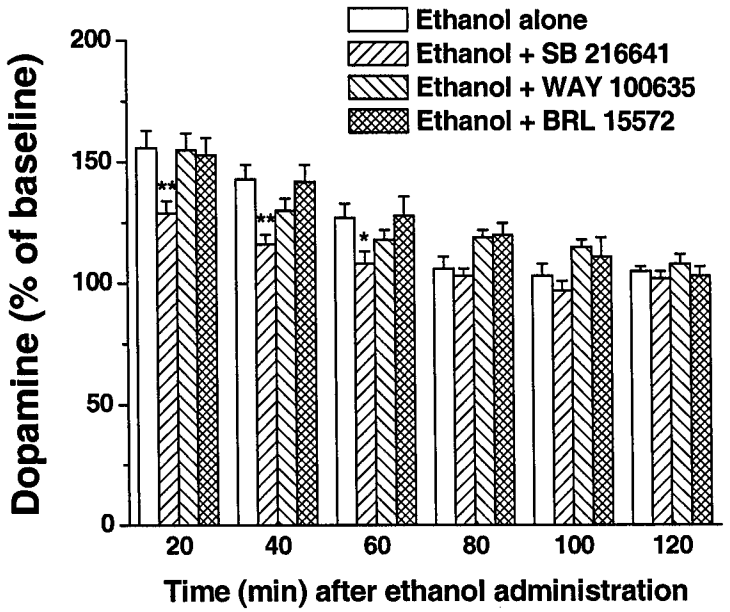

Fig 5. Comparisons of ethanol (2 g/kg)-induced VTA DA release in the presence and absence of SB 216641, WAY 100635, or BRL 15572. SB 216641 (10 μm), WAY 100635 (10 μm), or BRL 15572 (10 μm) was infused into the VTA 20 min before ethanol ip injection until the end of the experiments. Results are mean ± SEM from six to eight animals. The data of the ethanol alone group were obtained from Fig 1. * $P < 0.05$, ** $P < 0.01$ as compared with the ethanol alone group (two-way ANOVA followed by Tukey's tests). The basal DA levels (fmol/sample) in the VTA were: 8.94 ± 0.72 (the ethanol alone group, n=7), 8.24 ± 0.83 (the ethanol + SB 216641 group, n=8), 9.25 ± 1.04 (the ethanol + WAY 100635 group, n=7), and 9.06 ± 0.93 (the ethanol + BRL 15572 group, n=6).

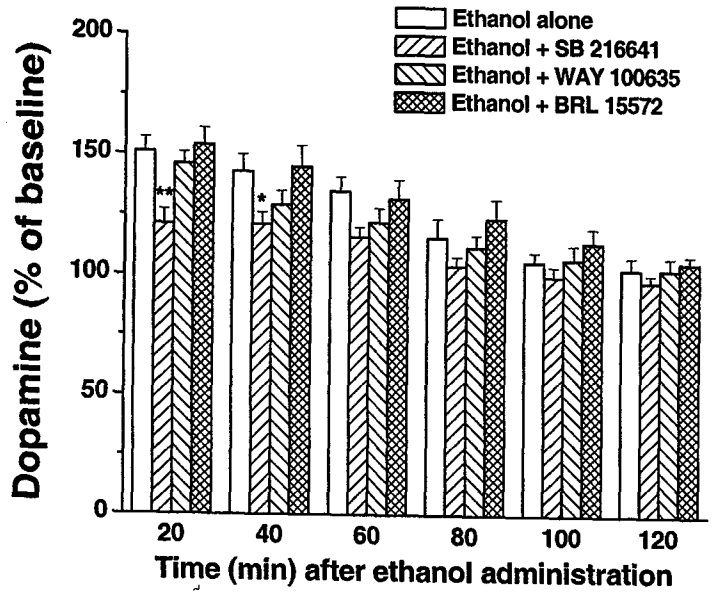

Fig 6. Comparisons of ethanol (1 g/kg)-induced NACC DA release in the presence and absence of SB 216641, WAY 100635, or BRL 15572. SB 216641 (10 μm), WAY 100635 (10 μm), or BRL 15572 (10 μm) was infused into the VTA 20 min before ethanol ip injection until the end of the experiments. Extracellular DA in the ipsilateral NACC was monitored by a second probe in this region. Results are mean ± SEM from seven to eight animals. The data of the ethanol alone group were obtained from Fig 2. * $P < 0.05$, ** $P < 0.01$ as compared with the ethanol alone group (two-way ANOVA followed by Tukey's tests). The basal DA levels (fmol/sample) were: 34.39 ± 2.72 (the ethanol alone group, n=7), 46.78 ± 4.84 (the ethanol + SB 216641 group, n=8), 36.92 ± 3.96 (the ethanol + WAY 100635 group, n=7), and 37.71 ± 3.47 (the ethanol + BRL 15572 group, n=7).

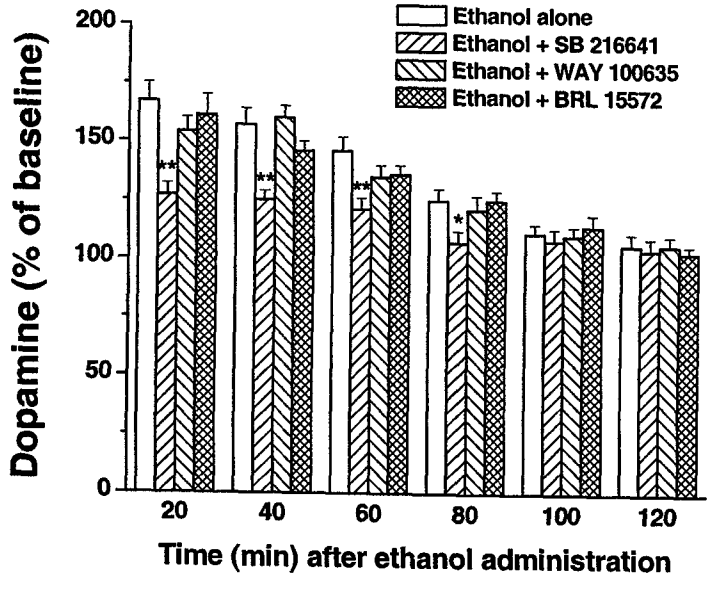

Fig 7. Comparisons of ethanol (2 g/kg)-induced NACC DA release in the presence and absence of SB 216641, WAY 100635, or BRL 15572. SB 216641 (10 μm), WAY 100635 (10 μm), or BRL 15572 (10 μm) was infused into the VTA 20 min before ethanol ip injection until the end of the experiments. Extracellular DA in the ipsilateral NACC was monitored by a second probe in this region. Results are mean ± SEM from six to eight animals. The data of the ethanol alone group were obtained from Fig 2. * $P < 0.05$, ** $P < 0.01$ as compared with the ethanol alone group (two-way ANOVA followed by Tukey's tests). The basal DA levels (fmol/sample) were: 28.24 ± 2.30 (the ethanol alone group, n=7), 26.83 ± 2.53 (the ethanol + SB 216641 group, n=8), 28.79 ± 2.20 (the ethanol + WAY 100635 group, n=7), and 27.64 ± 1.85 (the ethanol + BRL 15572 group, n=6).

3. Effects of infusion of CP 94253 into the VTA on ethanol (1 and 2 g/kg)-induced DA release in the ipsilateral NACC. Dual-probe microdialysis was employed the same as above except that CP 94253 (10 μM) was infused into the VTA 20 minutes before ethanol injection until the end of the experiments. CP 94253 was chosen because it has been found to have 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)[11]. In addition, CP 94253 has an advantage over CP 93129, which by itself can produce a peak under our experimental conditions that may sometimes interfere with the peak of DA. 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. In a group of rats, infusion of 10 μM of CP 94253 into the VTA for 140 min did not significantly alter extracellular DA levels in the ipsilateral NACC. The previous study also showed that infusion of CP 93129 into the VTA at the concentration of 20 μM did not significantly change extracellular DA in the ipsilateral NACC[1]. As shown in Figs 8 and 9, co-administration of CP 94253 significantly prolonged the 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. In the absence of CP 94253, extracellular DA in the NACC was increased rapidly to the maximum level after administration of ethanol at the doses of 1 (Fig 8) and 2 g/kg (Fig 9), then declined gradually 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 ethanol alone group at 80 – 120 min after administration of ethanol at the both doses (Figs 8 and 9).

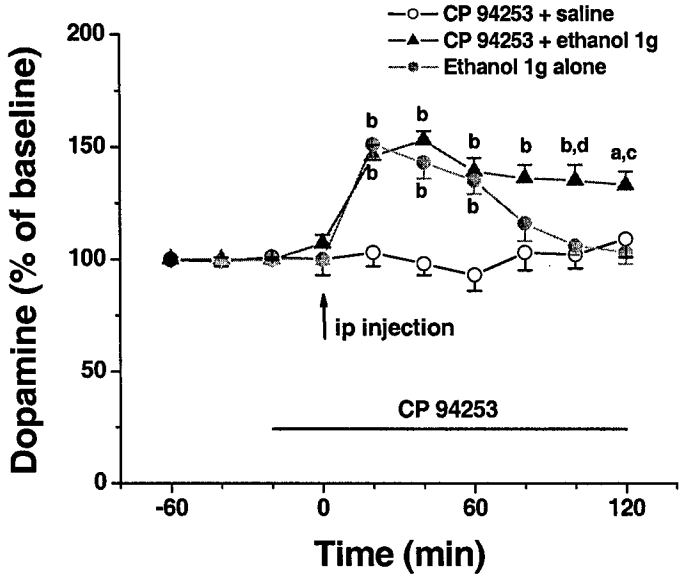

Fig 8. Effects of co-administration of CP 94253 on ethanol (1 g/kg)-induced NACC DA release. CP 94253 (10 μM) was infused into the VTA through a probe as indicated by the bar. Ethanol (1 g/kg) or saline was injected ip as indicated by the arrow. Extracellular DA in the ipsilateral NACC was measured by a second probe in this region. The data of the ethanol alone group was obtained from Fig 2. [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: 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 1 g/kg group).

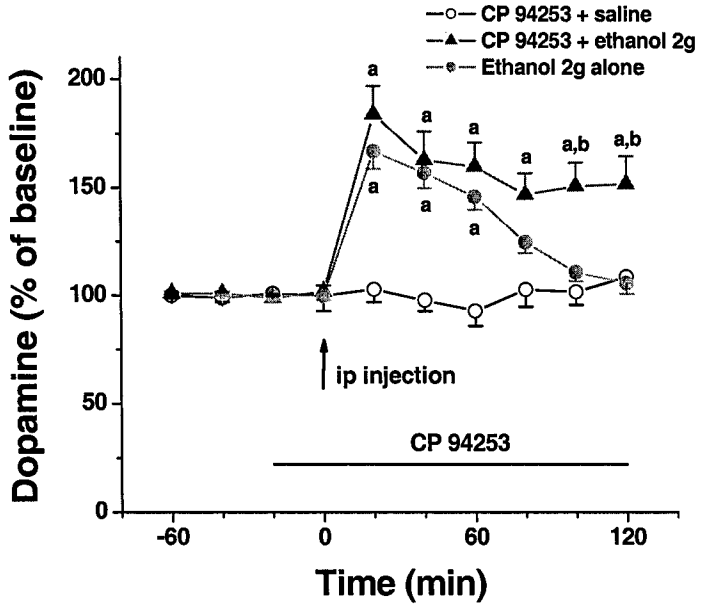

Fig 9. Effects of co-administration of CP 94253 on ethanol (2 g/kg)-induced NACC DA release. CP 94253 (10 μM) was infused into the VTA through a probe as indicated by the bar. Ethanol (2 g/kg) or saline was injected ip as indicated by the arrow. Extracellular DA in the ipsilateral NACC was measured by a second probe in this region. The data of the ethanol alone group was obtained from Fig 2. The data of the CP 94253 + saline group was obtained from Fig 8. [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: 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 2 g/kg group).

These results suggest that activation of VTA 5-HT-1B receptors by focally applied CP 94253 potentiates the effects of ethanol on the DA release in the ipsilateral NACC. Together with the data obtained with the antagonists, the results are consistent with the hypothesis under Specific Aim 1 – that is: Activation and blockade of VTA 5-HT-1B receptors potentiates and attenuates ethanol's effects on DA transmission in the ipsilateral NACC, respectively. The previous behavioral studies reported in the literature indicated that activation of 5-HT-1B receptors reduced ethanol intakes and reinforced behaviors in an operant paradigm[9,10]. Since increased dopaminergic transmission in the mesolimbic DA pathway has been implicated in the rewarding effects of ethanol, our observations may, at least in part, explain why 5-HT-1B receptors play a role in regulating ethanol rewarding effects and alcohol drinking behaviors. A manuscript for publishing all these data are currently under preparation.

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

According to the Statement of Work, Specific Aim 2 would be accomplished in approximately 18 months (Year 3 and the first half of Year 4). The proposed experiments would be carried out in both 5-HT-1B receptor knock-out (KO) mice and their counterparts wild type (WT) mice. There are three hypotheses that need to be tested under this aim. They are (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 transmission by activation of the 5-HT-1B receptor is absent in 5-HT-1B receptor knock-out mice.

As stated in the second annual report, due to a spread of mouse hepatitis virus at the vivarium of Dr. Rene Hen, a professor of Columbia University, who originally promised me to provide the KO and WT mice, I cannot obtain these animals directly from Dr. Hen. As a result, the work related to breeding of the KO and WT mice and genotyping of these animals were successfully developed at my laboratories during Year 2. However, breeding and genotyping substantially increased our workload, and consequently have impacts on the speed of the progress of the project.

4. Comparisons of the effects of RU 24969 on extracellular concentrations of DA in the NACC between the KO and WT mice. One-probe microdialysis was employed. After basal DA in the NACC was stable, saline or RU 24969, a 5-HT-1B/1A receptor agonist, was injected ip at the dose of 1 mg/kg to KO or WT mice. Extracellular DA concentrations in the NACC after saline or RU administration were measured and compared. The basal DA levels (fmol/sample) in the NACC were: 17.05 ± 2.99 (the KO saline group, n=6), 18.62 ± 2.03 (the KO RU 24969 group, n=6), 16.93 ± 2.53 (the WT saline group, n=6), and 17.45 ± 2.43 (the WT RU 24969 group, n=5). There were no statistically significant differences in the basal DA values between the KO and WT mice. As compared with saline injections, administration of RU 24969 did not produce significant changes in extracellular NACC DA concentrations in the KO mice (Panel A of Fig 10), but did so in the WT mice (Panel B of Fig 10). After RU 24969 administration, extracellular NACC DA concentrations were significantly higher at the time points of 60 – 140 min in the WT mice than those in the KO mice (panel C of Fig 10). The previous studies have shown that activation of 5-HT-1B receptors within the VTA increases DA release in the ipsilateral NACC in rats[1]. The present data showing that RU 24969 increased NACC DA in the WT but not in the 5-HT-1B receptor KO mice further support the notion that activation of 5-HT-1B receptors facilitates DA transmission in the mesolimbic DA pathway.

The other experiments under Specific Aim 2 are currently in progress. As mentioned in the previous annual reports, due to a delay in hiring the post-doctoral research associate in Year 1 and an unforeseen increase in workload resulting from breeding and genotyping of KO and WT mice in Years 2 and 3, the progress in the project has considerably lagged behind the originally proposed schedule. As a result, the PI may apply for a one-year no-cost extension.

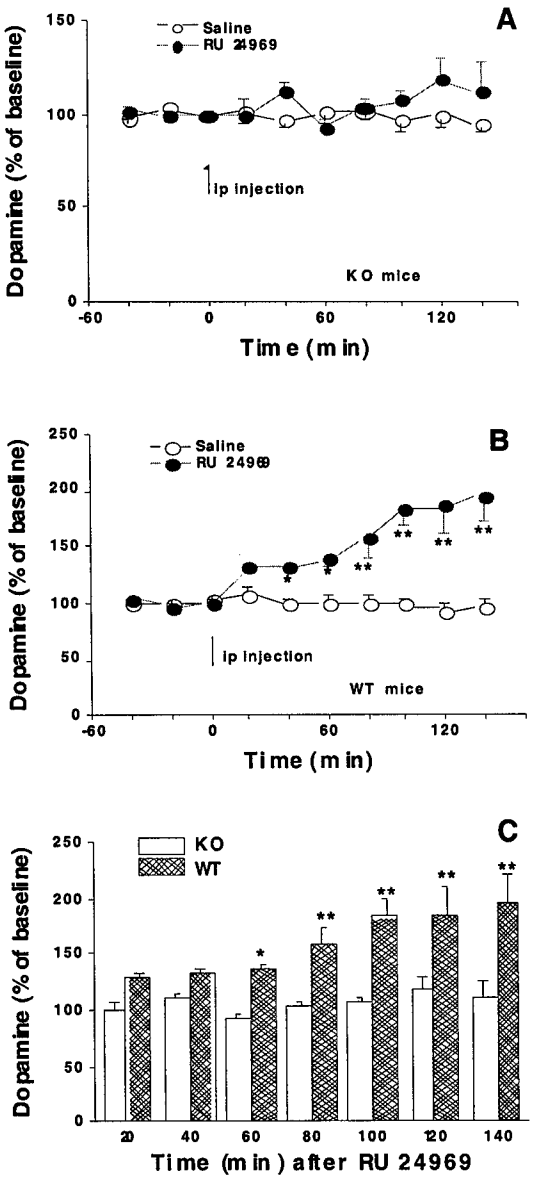

Fig 10. The effects of RU 24969 on extracellular DA concentrations in the NACC. Panels A and B show the effects in the KO and WT mice, respectively. RU 24969 (1 mg/kg) or saline was injected ip as indicated by the arrow. Results are mean ± SEM from five to six animals. * $P < 0.05$, ** $P < 0.01$ as compared with the saline group (two-way ANOVA followed by Tukey's tests). Panel C shows comparisons of the effects of RU 24969 on NACC DA between the KO and WT mice. * $P < 0.05$, ** $P < 0.01$ as compared with the KO mice (two-way ANOVA followed by Tukey's tests).

KEY RESEARCH ACCOMPLISHMENTS

1. We found that systemic administration of ethanol at the doses of 1 and 2 g/kg all increased extracellular DA concentrations not only in the VTA but also in the ipsilateral NACC. The time course of extracellular DA in the VTA after ethanol administration is similar to that in the NACC. Administration of ethanol at the same doses did not produce significant changes in extracellular GABA levels in the VTA.
2. Co-administration of the 5-HT-1B receptor antagonist SB 216641, but not the 5-HT-1A receptor antagonists WAY 100635 or the 5-HT-1D/1A receptor antagonist BRL 15572, into the VTA significantly attenuated systemic ethanol-evoked DA release in this region and in the ipsilateral NACC.
3. Co-administration of the 5-HT-1B receptor agonist CP 94253 into the VTA significantly prolonged the effects of ethanol on extracellular DA concentrations in the ipsilateral NACC.
4. The 5-HT-1B/1A receptor agonist RU 24969 increased extracellular NACC DA concentrations in the wild-type mice but not in the 5-HT-1B receptor knockout mice.

REPORTABLE OUTCOMES

Yan QS, Zheng SZ, Yan SE,: Involvement of 5-HT_{1B} receptors within the ventral tegmental area in regulation of mesolimbic dopaminergic neuronal activity via GABA mechanisms: a study with dual-probe microdialysis. Brain Res., 1021: 82-91, 2004 (please see Appendixes).

CONCLUSION

The results show that systemic administration of ethanol at the doses of 1 and 2 g/kg produces increases of DA release in both the VTA and the ipsilateral NACC in the same animal. The time course of DA release in the VTA after ethanol is similar to that in the NACC. The results suggest that increased somatodendritic and terminal DA releases are more likely due to ethanol-induced stimulation of mesolimbic DA neurons.

The blockade of 5-HT-1B receptors within the VTA attenuates not only ethanol-stimulated DA release in this region but also DA release in the ipsilateral NACC. The results suggest that VTA 5-HT-1B receptors are involved in ethanol-evoked stimulation of mesolimbic DA neurons. This conclusion is based on the data showing that ethanol-induced increases of the DA release in both areas were inhibited by the 5-HT-1B receptor antagonist but not by the 5-HT-1A or 5-HT-1D receptor antagonist. The mechanism by which VTA 5-HT-1B receptors are involved in ethanol-induced activation of VTA DA neurons remains to be illustrated. Although the previous studies showed that inhibition of VTA GABA release may contribute to the 5-HT-1B receptor-mediated activation of mesolimbic DA neurons, this mechanism may not be involved in ethanol's effects. This speculation is drawn from the data showing that no measurable changes in VTA GABA were detected after systemic ethanol administration.

Activation of VTA 5-HT-1B receptors potentiates the effects of ethanol on DA concentrations in the NACC. This conclusion is based on the data showing that infusion of CP 94253 into the VTA significantly prolonged ethanol-induced increases in extracellular DA concentrations in the NACC. Taken together, the present results are consistent with the hypothesis that activation and blockade of VTA 5-HT-1B receptors potentiates and attenuates ethanol's effects on DA transmission in the ipsilateral NACC, respectively. Our observations may in part explain why 5-HT-1B receptors play a modulatory role in ethanol's behavioral effects.

Systemic administration of the 5-HT-1B/1A receptor agonist RU 24969 increased extracellular DA concentrations in the NACC in the control animals but not in the 5-HT-1B receptor knockout animals. The results provide additional support to the hypothesis that 5-HT-1B receptors are involved in modulation of mesolimbic dopaminergic neurotransmission.

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Research report

Involvement of 5-HT_{1B} receptors within the ventral tegmental area in regulation of mesolimbic dopaminergic neuronal activity via GABA mechanisms: a study with dual-probe microdialysis

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Abstract

This study was designed to assess the involvement of 5-HT_{1B} receptors within the ventral tegmental area (VTA) in the regulation of mesolimbic dopaminergic transmission. 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). Drugs were administered into the VTA via retrograde dialysis. Dialysates from both the VTA and the NAC were collected for determination of dopamine (DA) and gamma-aminobutyric acid (GABA) by high-performance liquid chromatography with electrochemical detection. Intra-tegmental infusion of CP 93129 (20, 40, and 80 μM), a 5-HT_{1B} receptor agonist, increased extracellular DA concentrations in a concentration-dependent manner not only in the NACC but also in the VTA, indicating increased mesolimbic DA neuron activity. Administration of CP 93129 at 80 μM into the VTA also significantly decreased extracellular GABA concentrations in this region. Co-infusion of the 5-HT_{1B} receptor antagonist SB 216641 (10 μM), but not the 5-HT_{1A} receptor antagonist WAY 100635 (10 μM) or the 5-$HT_{1D/1A}$ receptor antagonist BRL 15572 (10 μM), antagonized not only the effects of intra-tegmental CP 93129 (80 μM) on VTA DA and NAC DA but also on VTA GABA. The results suggest that activation of VTA 5-HT_{1B} receptors increases mesolimbic DA neuron activities. The increased DA neuron activity may be associated, at least in part, with the 5-HT_{1B} receptor-mediated inhibition of VTA GABA release.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Serotonin receptors

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

1. Introduction

Anatomical studies of the afferent pathways to the ventral tegmental area (VTA) have indicated that this region receives serotonergic innervations from the dorsal and medial raphe nuclei [27,39]. Consistent with anatomical evidence, previous studies showed the existence of a functional relationship between 5-hydroxytryptamine (serotonin, 5-HT) and mesolimbic dopamine (DA) neurons within the VTA. Thus, direct administration of 5-HT [25] or the 5-HT_{1B} receptor agonist CP 93129 [64] {3-(1,2,5,6-tetrahydro-4-pyridyl)pyrrolo[3,2-*b*]pyrid-5-one} into the VTA has been found to increase extracellular DA concentrations in the ipsilateral nucleus accumbens (NACC) in vivo, suggesting activation of the mesolimbic DA neurons. Using intracellular recording technique, Pessia et al. [47] reported that 5-HT at concentrations of 3–100 μM depolarized VTA DA neurons in vitro.

The VTA contains DA and non-DA neurons [31]. Many of non-DA neurons are thought to be GABAergic, have been shown to contain GABA, the GABA synthetic enzyme glutamic acid decarboxylase, as well as glutamic

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acid decarboxylase mRNA [40,68]. Electrophysiological features of VTA GABA neurons have recently been characterized by Steffensen et al. [53] and Gallegos et al. [22], and found to be easily distinguished from those of VTA DA neurons. These VTA GABA neurons are presumed to modulate mesolimbic DA neuronal activity in a manner similar to the inhibition of nigrostriatal DA neurotransmission by substantia nigra pars reticulata GABA neurons [54].

It has been reported that 5-HT_{1B} receptors are predominantly located on axon terminals [4,51]. In keeping with this localization, 5-HT_{1B} receptors have been shown to regulate neurotransmitter release from nerve terminals. Activation of 5-HT_{1B} autoreceptors on serotonergic terminals resulted in inhibition of 5-HT release in the frontal cortex [19], hippocampus [50], and striatum [1]. Using electrophysiological techniques, Mlinar et al. [37,38] demonstrated that 5-HT_{1B} receptors were responsible for the presynaptic inhibition of neurotransmission in the CA1 region of the hippocampus. It has also been reported that 5-HT_{1B} receptors can function as a heteroreceptor in some brain areas to inhibit releases of glutamate [3], GABA [12,63] or acetylcholine [11]. Interestingly, recent studies showed that, in addition to 5-HT_{1A} receptors, 5-HT release in rat dorsal and median raphe nuclei was also controlled by 5-HT_{1B} autoreceptors [28] although the presence of 5-HT_{1B} receptors in the raphe nuclei has not been unequivocally demonstrated in histochemical studies [2].

There is a moderately high density of 5-HT_{1B} binding sites in the VTA [6]. A large proportion of 5-HT_{1B} receptors in the VTA is probably located on the terminal of GABAergic cells [7]. Using intracellular recording from midbrain DA neurons in a brain slice containing the VTA, Cameron and Williams [8,9] found that activation of 5-HT_{1B} receptors in the VTA by sumatriptan resulted in reductions of the magnitude of GABA-mediated inhibitory post-synaptic potential, suggesting that VTA 5-HT_{1B} receptors may be involved in modulation of GABA input into VTA DA neurons although the potential involvement of 5-HT_{1D} receptors cannot be ruled out. Our previous studies also showed that activation of 5-HT_{1B} receptors inhibited high potassium-evoked [^{3}H]GABA release from the VTA slices, supporting the hypothesis that 5-HT_{1B} receptors within the VTA can function as heteroreceptors to decrease GABA release in this region [63]. However, these studies were all performed in vitro. At present, direct in vivo evidence supporting the involvement of VTA 5-HT_{1B} receptors in the regulation of mesolimbic DA neuronal activities via GABA mechanisms is lacking.

The present study was designed to address this issue by investigating the role of VTA 5-HT_{1B} receptors for the modulation of DA and GABA release in the VTA and DA release in the ipsilateral NACC in the same animals. Towards this aim, dual-probe microdialysis was used with one in the VTA and the other in the ipsilateral NACC. Several serotonergic agents were administered alone or in combination into the VTA via retrograde microdialysis to minimize the effects of the compounds on the structures other than the VTA. The effects of the drugs on extracellular DA and GABA levels in the VTA and DA levels in the NACC were measured simultaneously.

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 (Indianapolis, IN, USA). They were housed at 21±3 °C, 40–60% relative humidity and were maintained under 12-h light/12-h 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-[diphenylmethyl]-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 93129 {3-(1,2,5,6-tetrahydro-4-pyridyl)pyrrolo[3,2]pyrid-5-one} 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. Other reagents used were of analytical grade.

2.3. Microdialysis

The animals were prepared for the microdialysis experiments as described in a previous paper [64]. In brief, surgery was conducted on a Kopf stereotaxic instrument under anesthesia with a combination of sodium pentobarbital (35 mg/kg, i.p.) and halothane (5% in oxygen). Dialysis guide cannulae (Harvard Apparatus, 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 1.0 mm, DV 8.0 mm according to the atlas of Paxinos and Watson [46]. The period of post-surgical recovery was at least 5 days. On the evening of the day before the experiment, 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 (I. D. 215±15 μm, molecular weight cut-off=6000; Spectrum Medical Industries, Los Angeles, CA, USA), were inserted while gently restraining the freely behaving rat. On the experimental day, ACSF, which contained (in mM) Na^+ (150), K^+ (3.0), Ca^{2+} (1.2), Mg^{2+} (0.8), Cl^- (155), was perfused at 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. Dialysate samples from the VTA were divided into two portions with one portion 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 [61,62,64]. All treatments were administered via a dialysis probe.

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) for the VTA samples or an ESA narrowbore column (MD-150×2/RP-C18, 3 μM) for the NACC samples, 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. For VTA DA determination, 10-μl dialysate samples were injected to ensure that the 5-μl injection loop was completely filled. For NACC DA assay, 20-μl dialysate samples were injected onto the column. 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, 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_2EDTA 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 ~50 pg at a 2:1 signal-to-noise ratio.

After completion of the dialysis, the animals were given an intracardiac perfusion with buffered saline and 10% formalin solutions under anesthesia with sodium pentobarbital, and then decapitated. 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 [46].

2.5. Data analysis

All values of DA and GABA reported herein represented uncorrected dialysate levels and were expressed as fmol/sample. The volume of the sample for determination of VTA DA, NACC DA, and VTA GABA was 5, 20, and 20 μl, respectively. 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 and appropriate accumbal DA responses to perfusion of the VTA with baclofen were included in data analyses. Approximately 75% of the animals that had undergone surgery had both probes correctly implanted in the VTA and NACC, and met the functional criterion.

3.1. Effects of infusion of CP 93129 into the VTA on extracellular DA concentrations in this region (Fig. 1) and in the ipsilateral NACC (Fig. 2)

Three concentrations of CP 93129 (20, 40 and 80 μM) were administered via a probe into the VTA of three groups of rats for 60 min, respectively, and extracellular levels of DA in both regions were monitored simultaneously. In another group of rats (the control group), ACSF was infused into the VTA for the same period as the drug groups and switching between syringes containing ACSF in this group was found to have no significant effects on the dialysate DA levels in the VTA (Fig. 1) or in the ipsilateral NACC (Fig. 2). As shown in Figs. 1 and 2, administration of CP 93129 produced concentration-dependent increases in extracellular DA levels in both the VTA and NACC. In both regions, infusion of 80 μM of CP 93129 caused more pronounced increases than 20 μM (P=0.015 and P=0.045 at 40 and 60 min in Fig. 1 and P=0.046 at 20 min after drug infusion in Fig. 2). The maximum increases of DA levels produced by 20, 40, and 80 μM of CP 93129 were 137%, 182%, and 242% of baseline in the VTA (Fig. 1), and 160%, 181%, 217% of baseline in the NACC (Fig. 2), respectively. The effects of 20 μM of CP 93129 on VTA DA did not reach statistical significance when compared with the control group.

3.2. Effects of infusion of CP 93129 into the VTA on extracellular GABA concentrations in this region (Fig. 3)

As shown in Fig. 3, infusion of ACSF alone (the control group) did not cause significant changes in GABA levels from VTA dialysates. Administration of CP 93129 at the concentration of 20 or 40 μM did not produce significant alterations in the level of VTA GABA either when compared with the control group although there were tendencies towards reductions following drug infusion. However, infusion of CP 93129 at the concentration of 80 μM caused extracellular GABA in the VTA to decrease by 37% of baseline (P=0.043 and P=0.04 at 20 and 60 min after drug application, respectively). Upon comparing the time course of VTA GABA with that of VTA DA or NACC

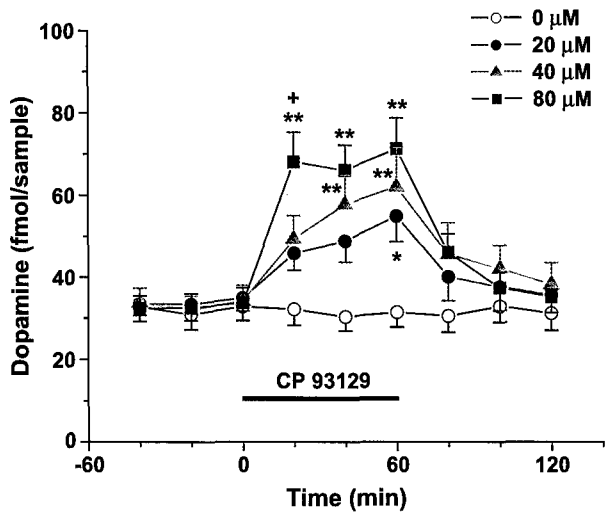

Fig. 2. Effects of local infusion of CP 93129 into the VTA on extracellular DA in the ipsilateral NACC. CP 93129 (20, 40, and 80 μM) was administered via the probe into the VTA during the period indicated by the bar. Extracellular DA in the ipsilateral NACC was monitored by a second probe in this region. Results are mean±S.E.M. from six to seven animals. *P<0.05, **P<0.01 as compared with the control (0 μM) group; +P<0.05 as compared with the 20-μM group (two-way ANOVA followed by Tukey's tests).

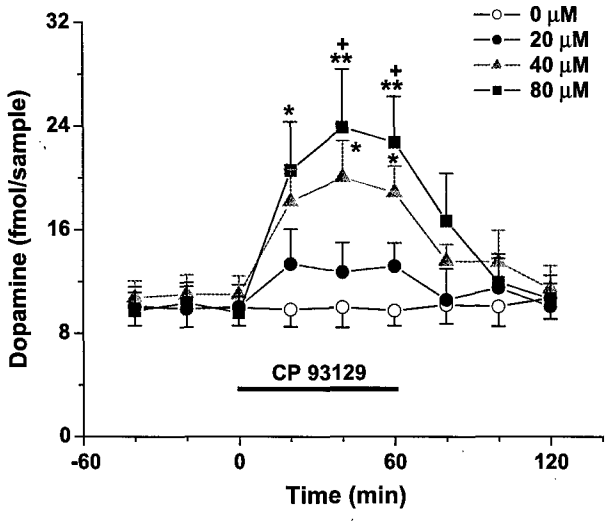

Fig. 1. Effects of local infusion of CP 93129 into the VTA on extracellular DA in this region. CP 93129 (20, 40, and 80 μM) was administered via the probe into the VTA during the period indicated by the bar. Results are mean±S.E.M. from six to seven animals. *P<0.05, **P<0.01 as compared with the control (0 μM) group; +P<0.05 as compared with the 20-μM group (two-way ANOVA followed by Tukey's tests).

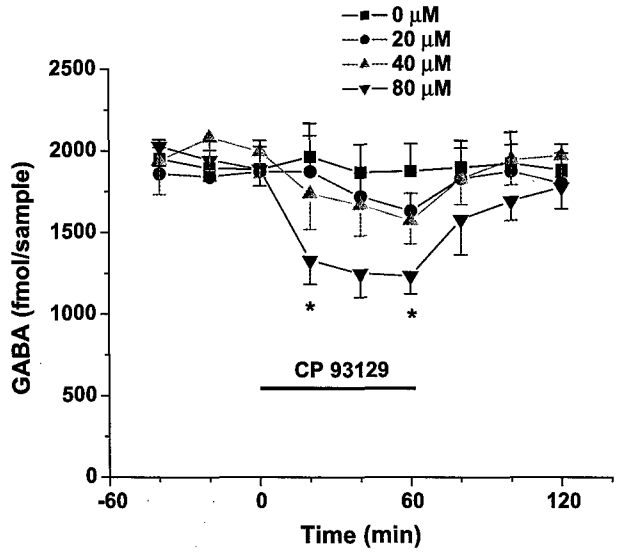

Fig. 3. Effects of local infusion of CP 93129 into the VTA on extracellular GABA in this region. CP 93129 (20, 40, and 80 μM) was administered via the probe into the VTA during the period indicated by the bar. Results are mean±S.E.M. from six to seven animals. *P<0.05 as compared with the control (0 μM) group (two-way ANOVA followed by Tukey's tests).

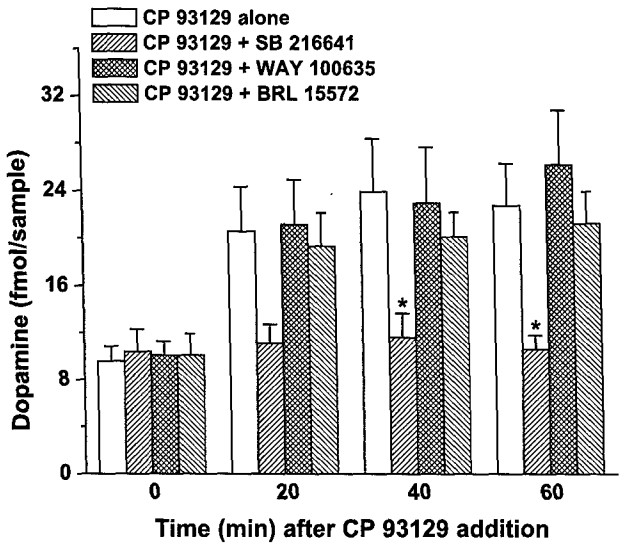

Fig. 4. Comparisons of CP 93129-induced increases of extracellular VTA DA in the presence and absence of WAY-100635, SB 216641, or BRL 15572. WAY-100635 (10 μM), SB 216641 (10 μM), and BRL 15572 (10 μM) were administered through the probe into the VTA for 40 min, and then co-infused with CP 93129 (80 μM) for another 60 min, respectively. Results are mean±S.E.M. from six to seven animals. The data of the group of CP 93129 (80 μM) alone were obtained from Fig. 1. *P<0.05 as compared with the group of CP 93129 alone (two-way ANOVA followed by Tukey's tests).

DA after administration of 80 μM CP 93129, we found that the decrease of VTA GABA was correlated temporarily with the increase of DA in both the VTA and the NACC.

3.3. Effects of WAY-100635, SB 216641, or BRL 15572 on intra-tegmental CP 93129 (80 μM)-induced DA release in the VTA (Fig. 4) and the ipsilateral NACC (Fig. 5)

In these experiments, WAY 100635 (a 5-HT_{1A} receptor antagonist), SB 216641 (a 5-HT_{1B} receptor antagonist), and BRL 15572 (a 5-$HT_{1D/1A}$ receptor antagonist) were used. All these drugs were infused into the VTA at the concentration of 10 μM for 40 min alone and then co-infused with CP 93129 (80 μM) for another 60 min, respectively. The concentration of 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 [21]. Our previous in vitro study showed that 0.45 μM SB 216641 completely antagonized 5-HT_{1B} receptor-mediated inhibition of [^{3}H]GABA release from rat VTA slices [63]. 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 [28].

In separate groups of rats infusion of WAY 100635, SB 216641, 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 (data not shown).

Figs. 4 and 5 show comparisons of intra-tegmental CP 93129 (80 μM)-induced accumbal DA release in the presence and absence of WAY-100635, SB 216641, or BRL 15572. It can be seen from these figures, the CP 93129-induced DA releases in the VTA and the NACC were all significantly attenuated by co-infusion of SB 216641. In the presence of SB 216641, CP 93129 (80 μM)-induced DA outputs in the VTA (Fig. 4) and NACC (Fig. 5) were all significantly lower than those in the absence of SB 216641 (P<0.05, two-way ANOVA followed by Tukey's tests). However, co-administration of WAY-100635 or BRL 15572 had no significant effects on the CP 93129-induced DA release in either the VTA or the NACC.

3.4. Effects of WAY-100635, SB 216641, or BRL 15572 on intra-tegmental CP 93129 (80 μM)-induced reductions of GABA release in the VTA (Fig. 6)

In separate groups of rats infusion of WAY 100635, SB 216641, or BRL 15572 at the same concentration used into the VTA for 2 h did not alter significantly extracellular GABA levels in the VTA (data not shown). As shown in Fig. 6, the basal VTA GABA values did not differ among the experimental groups. Administration of SB 216641, but not WAY 100635 or BRL 15572, antagonized the effects of intra-tegmental CP 93129 on VTA GABA.

At the end of each experiment, baclofen (50 μM) was infused into the VTA. Infusion of baclofen caused extracellular dopamine in the ipsilateral NACC to decrease to 30–

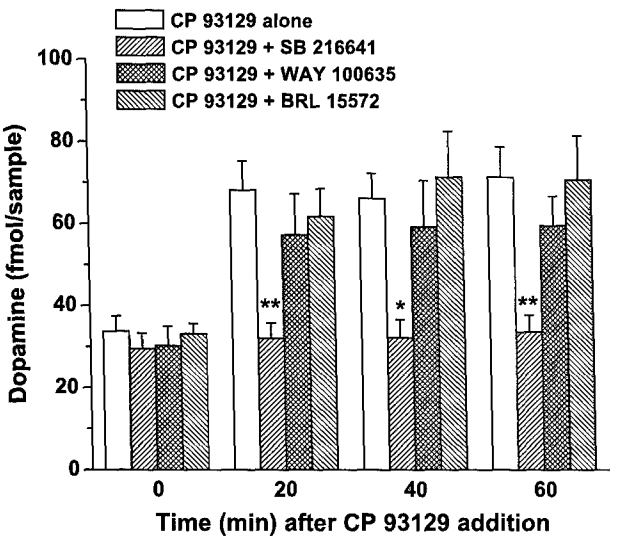

Fig. 5. Comparisons of CP 93129-induced increases of extracellular NACC DA in the presence and absence of WAY-100635, SB 216641, or BRL 15572. WAY-100635 (10 μM), SB 216641 (10 μM), and BRL 15572 (10 μM) were administered through the probe into the VTA for 40 min, and then co-infused with CP 93129 (80 μM) for another 60 min, respectively. Extracellular DA in the ipsilateral NACC was monitored by a second probe in this region. Results are mean±S.E.M. from six to seven animals. The data of the group of CP 93129 (80 μM) alone were obtained from Fig. 2. *P<0.05, **P<0.01 as compared with the group of CP 93129 alone (two-way ANOVA followed by Tukey's tests).

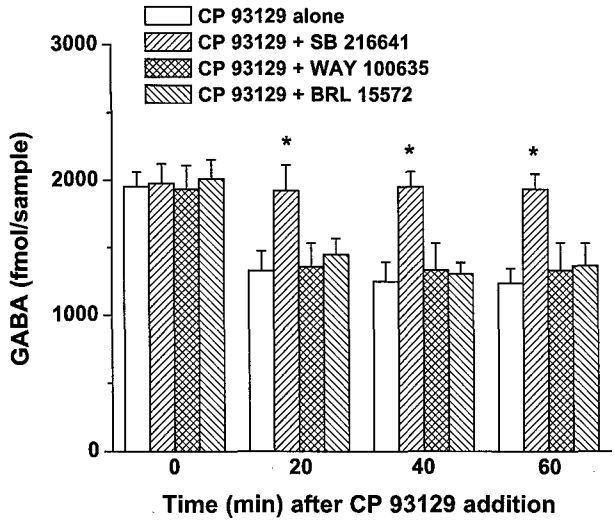

Fig. 6. Comparisons of CP 93129-induced decreases of extracellular VTA GABA in the presence and absence of WAY-100635, SB 216641, or BRL 15572. WAY-100635 (10 μM), SB 216641 (10 μM), and BRL 15572 (10 μM) were administered through the probe into the VTA for 40 min, and then co-infused with CP 93129 (80 μM) for another 60 min, respectively. Results are mean±S.E.M. from six to seven animals. The data of the group of CP 93129 (80 μM) alone were obtained from Fig. 3. *$P<0.05$ as compared with the group of CP 93129 alone (two-way ANOVA followed by Tukey's tests).

45% of baseline (data not shown). The observed effects of baclofen on NACC DA were consistent with those reported in the literature [57,67], suggesting the functional integrity of the circuitry studied in our dual-probe design.

4. Discussion

The DA release in both somatodendritic (VTA) and axon terminal areas (NACC) were simultaneously determined in this study. 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 [15,30]. Microdialysis findings have shown that, similar to extracellular DA in the NACC, extracellular DA in the VTA was tetrodotoxin sensitive and calcium dependent [10,13,32], suggesting that a depolarization-induced, exocytosis-mediated, somatodendritic release of DA occurred in the VTA under basal conditions. Evidence also indicated that extracellular DA in the VTA was regulated by D2 autoreceptors and DA reuptake processes [14,32]. Taken together, the data suggest that somatodendritic DA in the VTA, like that released from axon terminals in the NACC, was also dependent on the neuronal firing rate and thus can be used as an index of the activity of mesolimbic DA neurons. Drugs that increase the firing rate of mesolimbic DA neurons increase the extracellular DA levels in both the VTA and the NACC when they are focally applied into the VTA. For example, intra-tegmental administration of nicotine, a drug that increases mesolimbic DA transmission by augmentations of DA neuronal firing rates [36,41], increased both somatodendritic DA release in the VTA and synaptic DA release in the NACC [66]. However, administration of cocaine into the VTA has been found to decrease NACC DA while increasing VTA DA [16], a finding that was consistent with inhibitory effects of cocaine on DA cell firing mediated by the drug-induced increase of VTA DA. Therefore, determination of both somatodendritic and axon terminal DA release may provide more complete profiles of mesolimbic DA neuronal activities.

The present in vivo study shows that local application of CP 93129, a 5-HT_{1B} receptor agonist, into the VTA increased not only somatodendritic DA release in this region but also DA release from nerve terminals in the NACC, consistent with the excitation of mesolimbic DA neurons. CP 93129 is a putatively specific 5-HT_{1B} receptor agonist, with ≥150-fold higher affinity for 5-HT_{1B} vs. other 5-HT_1 and 5-HT_2 ligand binding sites (IC_{50} values: 5-HT_{1A} 3000±400, 5-HT_{1B} 15±5, 5-HT_{1D} 2200±700, and 5-HT_2> 10,000 nM) [17,35]. It is also claimed to lack substantial affinity for dopamine, noradrenaline or opiate receptors [35]. The concentrations of CP 93129 used in this study were 20–80 μM. Considering approximately ~5% of the efficiency of the probe used in the VTA, it is estimated that actual concentrations of CP 93129 in the extracellular fluid immediately adjacent to the dialysis membrane may be in the range of 1–4 μM. This poses the possibility that the selectivity of CP 93129 for the 5-HT_{1B} receptors may have been compromised. Although this possibility cannot be ruled out, two aspects of the dialysis technology may require the high concentrations. First, the rate constant of diffusion of compounds from the perfusion medium into the brain may be high enough that higher concentrations in the perfusate are necessary to maintain concentrations in the neuropil; these concentrations can be achieved at lower levels in in vitro preparations where a significant diffusion barrier is not present. Second, in addition to diffusion across the dialysis membrane, a concentration gradient away from the probe surface into the neuropil will exist. Thus, to achieve pharmacologically active concentrations in the neuropil not adjacent to the dialysis membrane it may be necessary to have higher drug concentrations in the tissue adjacent to the probe.

To assess the involvement of 5-HT_{1B} receptors in CP 93129's actions, WAY-100635 (a 5-HT_{1A} receptor antagonist) [21], SB 216641 (a 5-HT_{1B} receptor antagonist) [26,48], and BRL 15572 (a 5-$HT_{1D/1A}$ receptor antagonist) [26,48] were used. The affinities (given as pK_i) of these antagonists for 5-HT-$_{1A}$,-$_{1B}$, and-$_{1D}$ 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 [26,48]. All these drugs were tested for their impacts on the actions of CP 93129 at a highest concentration used, i.e., 80 μM, since, theoretically, higher concentrations of CP 93129 would have less selectivity as compared with lower concentrations. If 5-HT_{1A} receptors are involved in the actions of CP 93129, 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 CP 93129 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 intra-tegmental CP 93129 on extracellular DA in either the VTA or the NACC. These results are in opposition with the involvement of 5-HT_{1A} receptors in the observed CP 93129's effects.

Until recently, it has been impossible to distinguish pharmacologically between 5-HT_{1B} and 5-HT_{1D} receptors, but compounds have now been identified which show selectivity for both receptor subtypes. SB 216641 has been reported to have high affinity and selectivity for 5-HT_{1B} over 5-HT_{1D} receptors [48]. This drug shows more than 10-fold higher selectivity at 5-HT_{1B} (pK_i=9.0) receptors compared to 5-HT_{1D} (pK_i=7.6) [48]. 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 [48]. It has been shown that the hypothermic responses to the 5-$HT_{1B/1D}$ receptor agonist SKF-99101H [3-(2-dimethylaminoethyl)-4-chloro-5-propoxyindole hemifumarate] was dose-dependently blocked by SB 216641 but not by BRL 15572 [26]. It has also been reported that CP 93129 (0.3 μM)-induced inhibition of 5-HT release was antagonized by SB 216641 at concentrations of 0.05 and 0.2 μM but remained unaffected in the presence of BRL 15572 (0.5 μM) [28]. The present data show that intra-tegmental CP 93129-induced augmentations of VTA DA and NACC DA were antagonized by local infusion of 10 μM SB 216641. Considering approximately 5% of the probe efficiency, it could be estimated that the actual concentration of SB 216641 in the extracellular fluid may be in the range of ~0.5 μM. Therefore, inhibitory effects seen with SB 216641 might be afforded by its antagonism either at 1B or 1D or both receptor subtypes since, as mentioned above, the p*Ki* values of the drug for 1B and 1D receptor subtypes are 9.0 and 7.6. However, the results with BRL 15572 do not support the involvement of the 1D subtype in the actions of CP 93129. In the present study, BRL 15572 was administered at the same concentration (10 μM) as that of SB 216641. If antagonism at the 1D subtype makes a contribution to the observed effects of SB 216641, administration of BRL 15572 would also produce antagonistic effects which are similar in magnitude to those by SB 216641 since the former has similar or even higher affinity (pK_i=7.9) for the 1D subtype than does the latter (pK_i=7.6). However, the data presented in this study show that intra-tegmental CP 93129-induced augmentations of VTA DA and NACC DA were significantly antagonized only by local infusion of 10 μM SB 216641 but not by 10 μM BRL 15572. These data are consistent with the involvement of 5-HT_{1B} receptors but not 5-HT_{1D} receptors in CP 93129's actions. Taken together, the present results suggest that increased mesolimbic DA neuron activities following intra-tegmental administration of CP 93129 may be associated with the drug-induced activation of 5-HT_{1B} receptors within the VTA.

As mentioned in Introduction, The VTA contains DA and non-DA neurons [31]. Many of non-DA neurons are thought to be GABAergic, have been shown to contain GABA, the GABA synthetic enzyme glutamic acid decarboxylase, as well as glutamic acid decarboxylase mRNA [40,68]. Neuropharmacological findings indicate that VTA DA neurons appear to receive tonic inhibitory inputs from these GABAergic neurons and from descending GABA-containing terminals originated from the NACC and the globus pallidus [33,59]. Using the reverse transcription-polymerase chain reaction technique, Okada et al. [42] observed that various $GABA_A$ receptor subunits were expressed in VTA DA neurons. The blockade of $GABA_A$ receptors within the VTA with focal application of bicuculline [57] or picrotoxin [29] has been reported to increase extracellular DA concentration in the ipsilateral NACC, suggesting the augmentation of mesolimbic DA neuronal activity.

The present study shows that local infusion of CP 93129 at the concentration of 80 μM caused a decrease of extracellular GABA in the VTA. This effect of CP 93129 was also antagonized by local administration of SB 216641 but not by either WAY 100635 or BRL 15572. The results suggest that it is activation of VTA 5-HT_{1B} receptors that may be associated with the drug-induced reduction of GABA release in this region. These data are in good agreement with our previous in vitro studies showing that activation of 5-HT_{1B} receptors by CP 93129 or RU 24969 inhibited high potassium-evoked [^{3}H]GABA release from rat VTA slices in a concentration-dependent fashion [63]. Chadha et al. [12] also reported that activation of 5-HT_{1B} receptors by CP 93129 (0.6–16.2 μM) produced a concentration-dependent inhibition of 25 mM KCl-evoked [^{3}H]GABA release from slices of the rat globus pallidus. Since VTA DA neurons are under inhibitory GABAergic controls as mentioned above, reductions of VTA GABA release by 80 μM of CP 93129 may account for the drug-induced increase of VTA DA neuronal activity. That is, intra-tegmental infusion of CP 93129 may act at 5-HT_{1B} heteroreceptors to decrease GABA release in the VTA. The reduction of inhibitory GABAergic input to the VTA DA neurons would lead to disinhibition of these neurons, consequently resulting in increases in both somatodendritic and axon terminal DA release.

The present study shows that administration of CP 93129 at lower concentrations (i.e., 20 or 40 μM) did not significantly alter GABA levels in dialysates from the VTA but did increase extracellular DA in the VTA and the NACC. The results suggest that, in addition to indirect disinhibition of VTA DA neurons resulting from decreased GABAergic control, other mechanisms may also be involved in CP 93129-induced augmentations of mesolimbic DA transmission. On the other hand, previous microdialysis studies indicate that the part of GABA as measured

by microdialysis does not fulfill the classical criteria for exocytotic release [55], suggesting that GABA levels monitored by microdialysis probes may derive from non-neuronal pools in addition to the neuronal origin. Therefore, it is possible that decreased exocytotic GABA release resulting from lower concentrations of CP 93129 (i.e., 20 or 40 μM) may only cause a small change in total extracellular GABA levels that cannot be detected by our HPLC system.

We observed that administration of 10 μM SB 216641 alone into the VTA for 2 h did not significantly alter basal GABA concentrations in this region (data not shown). This finding may be reflective of the limits of detection as mentioned above. However, this finding may also suggest that 5-HT_{1B} receptors within the VTA are not involved in the modulation of GABA levels in this region during normal tonic or basal conditions. This speculation is in agreement with the finding that administration of 10 μM SB 216641 alone into the VTA did not significantly alter basal VTA DA or NACC DA either (data not shown).

The interaction between 5-HT and DA via the 5-HT_{1B} receptor within the VTA may play an important role in several behavioral disorders such as drug abuse. 5-HT_{1B} receptors have been shown to enhance the reinforcing properties of both cocaine and the selective dopamine reuptake inhibitor GBR 12909 [43,52]. Recently, Filip et al. [20] showed that intra-tegmental microinjection of the 5-HT_{1B} receptor agonist and antagonist increased and decreased the discriminative stimulus effects of cocaine, respectively, suggesting that the tegmental 5-HT_{1B} receptors were necessary for full expression of cocaine discrimination. Infusion of CP 93129 into the VTA also dose-dependently potentiated cocaine-induced increases of NACC DA efflux and motor activation in cocaine-naïve rats [45]. Studies carried out in mice lacking 5-HT_{1B} receptors indicated that 5-HT_{1B} receptors are important for ethanol's rewarding effects [18,49]. It has been demonstrated that VTA DA neurons play a critical role in the reinforcing/rewarding properties of many drugs of abuse [34]. Since inhibition of GABA release within the VTA may lead to activation of VTA DA neurons by disinhibition, this increased activity of VTA DA neurons may be associated with 5-HT_{1B} receptor-mediated regulation of rewarding effects of abused drugs. In fact, there is a report in the literature showing that the potentiation of cocaine reinforcement by the 5-HT_{1B} receptor agonist was mediated by a potentiation of increases of mesolimbic DA transmission via GABA mechanisms [44]. Investigation of the interaction among 5-HT, DA and GABA within the VTA via the 5-HT_{1B} receptor would provide further insight into mechanisms regulating behavioral responses to abused drugs, thereby, providing for alternative approaches to treatment of drug addiction. For example, ethanol has been reported to stimulate mesolimbic DA neurons [5,23] leading to increased DA release in the VTA [10] and the NACC [60]. Local infusion of ethanol also increased extracellular 5-HT concentrations in the VTA [65]. The investigation of possible involvement of VTA 5-HT_{1B} receptors in the regulation of the effects of ethanol on the mesolimbic DA neuronal activity may explain why 5-HT_{1B} receptors are important for modifying the reinforcing [49], discriminative stimulus effects [24], and voluntary intakes [56,58] of ethanol.

In summary, the present results suggest that activation of VTA 5-HT_{1B} receptors is associated with CP 93129-induced activation of mesolimbic DA neurons. This conclusion is based on the fact that intra-tegmental CP 93129 increased DA release in both the VTA and the NACC and that the increase of the DA release was antagonized by the 5-HT_{1B} receptor antagonist but not by the 5-HT_{1A} or 5-HT_{1D} receptor antagonist. The results also suggest that the 5-HT_{1B} receptor-mediated inhibition of VTA GABA release may contribute to the observed activation of mesolimbic DA neurons. This conclusion is drawn from the fact that intra-tegmental CP 93129 concomitantly caused a reduction of VTA GABA, an effect that was also blocked by the 5-HT_{1B} receptor antagonist. In addition to this indirect disinhibition of VTA DA neurons resulting from decreased GABAergic control, other mechanisms may also be involved in CP 93129-induced augmentation of mesolimbic DA transmission, particularly under lower concentrations of the drug. This speculation is based on the fact that CP 93129 increased DA release at all concentrations tested but significantly decreased VTA GABA release only at a higher concentration.

Acknowledgments

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