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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

According to Statement of Work, Hypothesis 1 under Specific Aim 1 was tested during Year 1.To this end, dual-probe microdialysis was employed and the 5-HT1B receptor agonist CP 93129 used. The experiments were performed in freely-moving rats. One probe was in the ventral tegmental area (VTA) and the other in the ipsilateral nucleus accumbens (NACC). Artificial cerebrospinal fluid (ACSF) alone and ACSF with three concentrations of CP 93129 (20, 40, and 80 μM) were infused respectively into the VTA of separate groups of rats for 60 min. Dialysates from the VTA and NACC were collected at 20-min intervals for determination of NACC DA and VTA GABA via HPLC systems. The results showed that administration of CP 93129 caused significant increases of extracellular DA concentrations in the NACC in a concentration-related manner. The maximum increases of NACC DA produced by 20, 40, and 80 μM of CP 93129 were 163%, 181%, and 214% of baseline, respectively. The determination of GABA levels in the VTA is presently under way and has not been completed yet. The results suggest that activation of VTA 5-HT1B receptors by focally applied CP 93129 increases DA transmission in the ipsilateral NACC.

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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 in 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 mice and their counterparts wild-type mice; and (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

There are two hypotheses Under Specific aim 1: one is that activation of 5-HT_{1B} receptors in the VTA decreases GABA release in this area and increases DA transmission in the ipsilateral NACC and the other is that 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, Hypothesis 1 was supposed to be completed at the end of Year 1. However, due to unexpected circumstances, the progress of the project has considerably lagged behind what had been originally proposed. This delay is caused mainly by search for a post-doctoral research associate. According to our university's policies, the search for that person could not be initiated until the funds were awarded. In addition, the search was a time-consuming process. As a result, the post-doctoral research associate did not participate in this project until August of 2002, a half year later than the proposed date. Therefore, a delay in hiring the post-doctoral research associate has caused a considerably delay in the progress of the project.

Hypothesis 1 consists of two parts of experiments. One is associated with the 5-HT1B receptor agonist CP 93129. This part of experiments have almost been accomplished during Year 1. Due to the delay in the progress mentioned above, the second part of experiments will be postponed to Year 2. In the first part of experiments, the dual-probe microdialysis, a technically very difficult procedure, was used in awake and freely-moving adult Sprague-Dayley rats. One probe was inserted into the VTA and the other in the ipsilateral NACC. Both probed were perfused with artificial cerebrospinal fluid (ACSF). After basal DA release in the NACC was stable, ACSF alone and ACSF with three different concentrations of CP 93129 (20, 40, and 80 μM), a selective 5-HT1B receptor agonist[1,2], were infused respectively into the VTA of separate groups of rats for 60 min. The dialysates from both VTA and the NACC were collected at 20 min of intervals for determination of DA in the NACC and gamma-aminobutyric acid (GABA) in the VTA via HPLC systems. The basal DA levels in the accumbal extracellular fluids were (fmol/sample, mean ± SEM): 33.15 ± 4.75 (the control group, n = 7), 34.94 ± 3.75 (the 20 μM group, n = 6), 33.62 ± 3.57 (the 40 μM group n = 7), and 32.99 ± 3.48 (the 80 μM group, n = 7). As shown in the following figure, switching between syringes containing ACSF has no significant effects on the dialysate DA levels in the ipsilateral NACC. However, administration of CP 93129 through a dialysis probe into the VTA caused significant increases of extracellular DA concentrations in the NACC in a concentration-related manner. The maximum increases of NACC DA produced by 20, 40, and 80 μM of CP 93129 were 163%, 181%, and 214% of baseline, respectively. The determination of GABA levels in the VTA is presently under way and has not been completed yet. Increased NACC DA after intra-tegmental CP 93129 is consistent with studies reported in the literature[3,4].

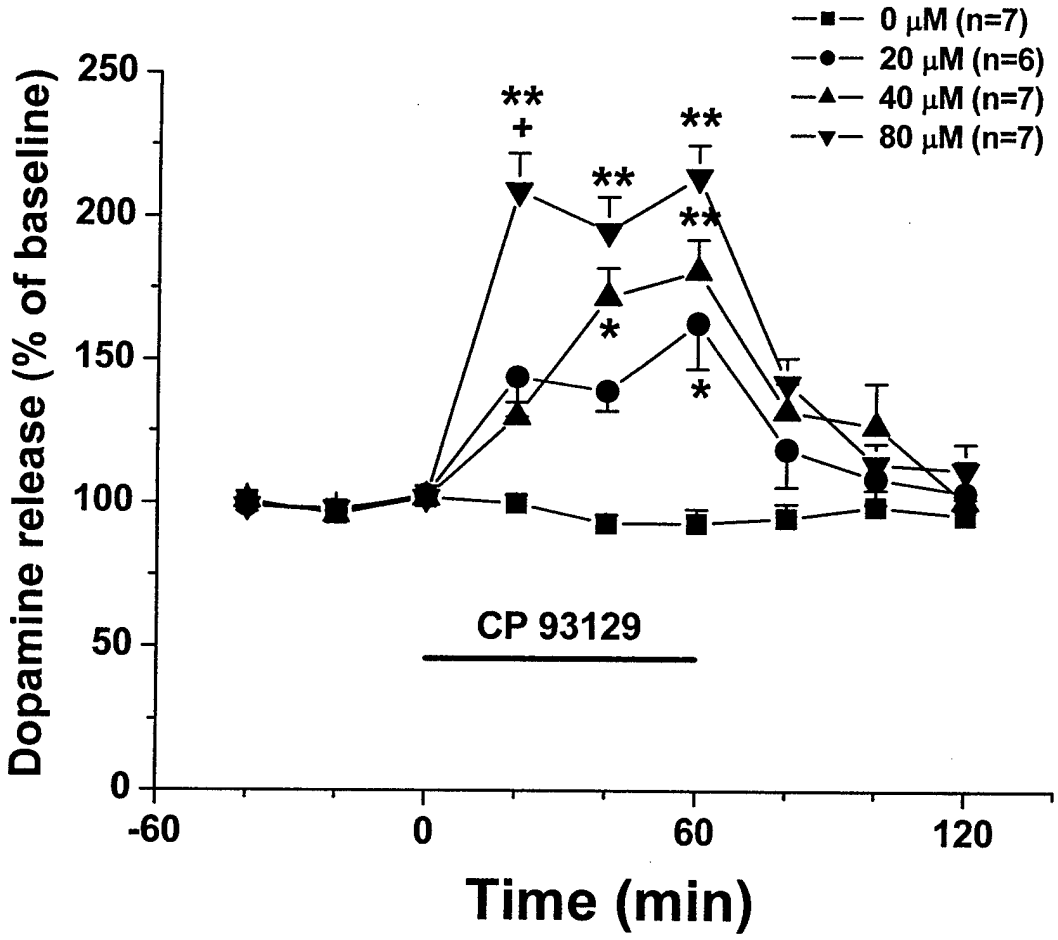

Fig. Effects of infusion of CP 93129 into the ventral tegmental area (VTA) on dopamine concentrations in the ipsilateral nucleus accumbens (NACC). CP 93129 (20, 40, and 80 μM) was administered through a probe into the VTA indicated by the bar. Extracellular dopamine in the ipsilateral NACC was monitored by a second probe in this region. Results are mean ± S.E.M. * $P < 0.05$, ** $P < 0.05$ as compared with the control (ACSF alone) group; + $P < 0.05$ as compared with the 40 μM group (two-way ANOVA followed by Tukey test).

KEY RESEARCH ACCOMPLISHMENTS

1. We have developed a dual-probe microdialysis method that is technically very difficult.
2. We have established a dose-response of the 5-HT1B receptor agonist CP 93129 on DA. We found that administration of CP 93129 into the VTA via a dialysis probe increased DA concentrations in the ipsilateral nucleus accumbens.

REPORTABLE OUTCOMES

None

CONCLUSIONS

The results obtained during Year 1 suggest that activation of VTA 5-HT1B receptors by focally applied CP 93129 increases DA transmission in the ipsilateral NACC. However, a firm conclusion could be drawn only after experiments with 5-HT1B receptor antagonists (i.e., the second part of experiments under Hypothesis 1) are completed.

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APPENDICES

None