Published in final edited form as:

Neuropharmacology. 2007 June ; 52(8): 1570–1579. doi:10.1016/j.neuropharm.2007.03.001.

Blockade of GABA_A Receptors within the Extended Amygdala Attenuates D₂ Regulation of Alcohol-Motivated Behaviors in the Ventral Tegmental Area of Alcohol-Preferring (P) Rats

William JA Eiler II³ and Harry L. June^{1,2}

¹ Division of Alcohol and Drug Abuse, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21201

² Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201

³ Psychobiology of Addictions Program, Department of Psychology, Indiana University-Purdue University, Indianapolis, IN 46202

Abstract

The dopamine (DA) mesolimbic pathway, which originates from DA cell bodies within the ventral tegmental area (VTA), has been shown by various studies to play a role in the mediation of various drugs of abuse including alcohol (EtOH). It has been suggested that the VTA's control of EtOH reward is mediated in part by the D₂ receptors within the VTA. These receptors may be under the regulation of reciprocal GABAergic inputs from forebrain components of the mesolimbic path such as the nucleus accumbens (NAcc), a classic EtOH reward substrate, and the bed nucleus of the stria terminalis, a substrate recently implicated in EtOH reinforcement, forming a self-regulating feedback loop. To test this hypothesis, D₂ regulation of EtOH self-administration (SA) was evaluated by the microinfusion of the D₂ antagonist eticlopride into the VTA of P rats, which produced profound reductions in EtOH SA in the highest (20.0 and 40.0 µg) doses tested in both BST/VTA and NAcc/VTA implanted P rats. To determine the role of GABA in the mediation of EtOH SA, a 32.0 ng dose of the non-selective GABA antagonist SR 95531 was microinfused into the BST producing no effect on responding for EtOH and into the NAcc which lead to a reduction in EtOH responding. Finally, the hypothesis that GABA innervation of the VTA from the mesolimbic forebrain may influence EtOH SA was examined by the simultaneous infusion of eticlopride (40.0 µg) into the VTA and SR 95531 (32.0 ng) into either the BST or NAcc. This combination infusion completely attenuated the reduction in EtOH SA observed with the 40.0 µg dose of eticlopride alone in both groups of animals. These results suggest that while the D₂ receptors within the VTA regulate EtOH-motivated behaviors, this is modulated by GABAergic input from the mesolimbic forebrain, specifically from the BST and NAcc.

Keywords

GABA_A; dopamine receptor; bed nucleus of the stria terminalis; nucleus accumbens; ventral tegmental area; SR 95531; Eticlopride; EtOH self-administration; P rats

Correspondence should be addressed to Harry L. June, Ph.D., Department of Psychiatry, University of Maryland Medical School, 701 West Pratt Street, Baltimore, MD 21201. Telephone: 410-706-4001 (voice). hjune@psych.umaryland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Considerable literature has implicated brain loci innervated by the DA mesolimbic path in the mediation of EtOH reward (Eiler et al., 2003; Melendez et al., 2004; Rassnick et al., 1992; Samson et al., 1993; for review see June and Eiler, in press; Samson et al., 1992). Originating from DA cell bodies located within the ventral tegmental area (VTA) the mesolimbic path travels anteriorly via the medial forebrain bundle (MFB) to innervate several limbic structures including the nucleus accumbens (NAcc), the bed nucleus of the stria terminalis (BST), the central nucleus of the amygdala (CeA), the hippocampus and the ventral pallidum (VP) (Oades and Halliday, 1987). Various studies have implicated all of these limbic structures innervated by the DA mesolimbic path in the mediation of EtOH reward (Eiler et al., 2003; Hodge et al., 1995; Hyttiä and Koob, 1995; June et al. 1998a; 2001).

Of the limbic loci, the NAcc is the most heavily researched in relation to EtOH reinforcement (for a recent review see Gonzales et al., 2004). Such a focus has been warranted by the finding that administration of ethanol consistently leads to increased release of extracellular dopamine within the NAcc (Di Chiara and Imperato, 1988; Imperato and Di Chiara, 1986; Yoshimoto et al., 1992). Many studies have also demonstrated that direct manipulation of the DA system within the NAcc leads to changes in the reinforcing qualities of EtOH itself. For example, the non-selective DA agonist amphetamine has been shown to increase EtOH responding in an operant self-administration paradigm when directly administered in to the NAcc of rats (Hodge et al., 1992, Samson et al., 1993). Hodge and colleagues (1997) examined the effects of selective agonists administered directly in the NAcc on EtOH responding and found that while the selective D1-like agonist SKF 38393 failed to alter EtOH responding the D2-like agonist quinpirole successfully reduced responding for EtOH. The same study examined the effects of a D1-like and D2-like antagonist on EtOH self-administration and found that both the D1-like antagonist SCH 23390 and the D2-like antagonist raclopride reduced EtOH responding when they were administered directly into the NAcc (Hodge et al., 1997). Various laboratories have also reported similar findings in which DA antagonists administered directly into the NAcc led to decreased EtOH intake or operant responding for EtOH (Hodge et al., 1994; Rassnick et al., 1992; Samson et al., 1993). The above findings all implicate the DA system of the NAcc in the regulation of EtOH reward.

When compared with the NAcc, only limited attention has been given to dopamine's role in other limbic structures. Research by Carboni and colleagues (2000) found that increased extracellular dopamine release similar to that often seen in the NAcc was found to occur in the BST following the administration in EtOH. This study suggests a role of the DA system within the BST in the mediation of EtOH reward. Research conducted in our laboratory has also elucidated such a role of the BST in the regulation of EtOH reward. It was shown that the D1-like antagonist SCH 23390 led to a dose dependent reduction in EtOH responding while the D2-like antagonist eticlopride produced no effect on EtOH reward (Eiler et al., 2003).

While the VTA is the point of origin for the mesolimbic pathway (Kalivas et al., 1993), few studies have investigated the effect of DA agents into the VTA on EtOH reward. In a 1993 study by Hodge et al., they reported that the D2 agonist quinpirole produced a dose dependent reduction on EtOH responding. Given that quinpirole inhibits DA cell activity in the VTA via a proposed feedback of the cell upon itself (Jeziorski and White, 1989), it would be predicted that quinpirole produced effects similar to blockade of DA neurotransmission. Indeed, Nowak et al., (2000) demonstrated that VTA infusions of quinpirole as well as quinlorane [a second D2 agonist] were both effective in reducing homecage alcohol, but not saccharin drinking in P rats. However, a selective reduction on alcohol intake was observed in the anterior, but not

the posterior VTA, suggesting that only the D₂ receptors on cell bodies within the anterior VTA selectively regulate alcohol intake (Murphy et al., 2002).

The effects of DA antagonists on EtOH reward have also been evaluated in the VTA. Samson and Chappell (1999) reported that the D₂ antagonist raclopride microinfused into the VTA reduced EtOH responding. This finding, however, was not confirmed in P rats, as the D₂ antagonist sulpiride failed to alter EtOH intake (Nowak et al., 2000). It is likely that differences in cannula placement may explain these discrepant results; however, given the paucity of research into the effects of centrally administered DA agents into the VTA, and the conflicting evidence as to their effects on EtOH reward, particularly D₂ antagonists, additional studies are warranted.

In addition to DA, previous research suggests a critical role for GABAergic neurotransmission in mediating alcohol reinforcement in the mesolimbic circuitry (see June and Eiler, in press). This is indicated by a number of studies that demonstrate changes in EtOH self-administration following the administration of various GABA agents. Several studies from both our lab and others have shown that administration of benzodiazepine inverse agonists (Ro 15-4513 and Ro 19-4603) can lead to reductions in the self-administration of EtOH during both limited access and operant self-administration paradigms (June et al., 1994a; 1994b; 1998b; McBride et al., 1988; Rassnick et al., 1993; Samson et al., 1987; Wegelius et al., 1994). Hyttiä and Koob (1995) conducted one of the key studies to elucidate GABAergic regulation in the mesolimbic path on alcohol-motivated behaviors. Employing the nonselective GABA_A receptor antagonist SR 95531, they reported that it was effective in reducing EtOH responding in the NAcc, BST, and CEA. Moreover, the effects seemed most selective for the CEA. More recent work, however, using benzodiazepine agonist-antagonists (e.g., 3-PBC and β CCt), have demonstrated that the CEA (Foster et al., 2004), and VP (Harvey et al., 2002; June et al., 2003) are important GABAergic regulators of alcohol-motivated behaviors.

Taken together, the above studies demonstrate that within various limbic loci, both DA and GABAergic mechanisms can regulate alcohol-reinforced behaviors. Thus, it is possible that a direct interaction may exist between the DA and GABAergic mechanisms within the mesolimbic system to regulate alcohol reward. It should be noted that neuroanatomical evidence exists showing that the forebrain loci of the mesolimbic path send reciprocal projections to the VTA (Sun and Cassell, 1993; Kalivas et al., 1993). While only the NAcc has been shown to send direct GABAergic projections to the VTA (Kalivas et al., 1993), it has been suggested that the BST may also send such projections to the VTA (Georges and Aston-Jones, 2002). In addition, there is evidence that a large number of GABA interneurons exist within these loci (Sun and Cassell, 1993). Thus, manipulation of the interneuronal GABAergic populations in these substrates may result in changes downstream within the VTA DA neurons which regulate alcohol-motivated behaviors.

The purpose of this study was threefold. This first goal was to address conflicting reports of a D₂ receptor regulatory control of EtOH reinforcement within the VTA. Secondly, the study attempted to address the role of GABAergic mechanisms of the BST and NAcc on EtOH-maintained responding. The final objective was to determine if GABA_A receptors of the BST or NAcc would modulate DAergic activity via the D₂ receptor in the VTA to alter alcohol responding.

MATERIALS AND METHODS

Subjects

Male and female alcohol-preferring (P) rats were used in the study. At the beginning of the study the rats weighed between 100 – 120g. Alcohol preferring (P) rats were used because of

their propensity to freely self-administer pharmacologically relevant levels of alcohol, and their general acceptance in the alcohol community as being an acceptable model of human alcohol drinking (McBride and Li, 1998). Also these animals are generally accepted as a genetic model of alcoholism in humans. All animals were individually housed in plastic cages in a vivarium at 21°C on a 12h light/dark cycle. Food and water were provided ad libitum for the animals, except for the conditions noted under the training phase.

Apparatus

Animals were tested in 15 standard operant chambers (Coulbourn Instruments, Inc, Lehigh Valley, PA) equipped with 2 levers and 2 dipper assemblies. Red, yellow and green cue lights were used to indicate the presence of a reinforcer. Each reinforced response delivered 0.1 ml of the reinforcer. Operant sessions were 30 minutes.

Drugs and Solutions

The EtOH (10% v/v) drinking solution was produced by mixing 100 ml of 100% EtOH with 900 ml of deionized water. Eticlopride and SR 95531 were mixed immediately before the experiments and were injected **unilaterally** using a Harvard Pump. The eticlopride and SR95531 mixed readily with 1 ml of sterile saline. The eticlopride and SR95531 were purchased from Sigma Drug Company (St. Louis, MO).

Behavioral Training Phase

Rats were trained to lever press for EtOH (10 % v/v) using a modified version of the sucrose fading-technique (June et al., 2001). For all training protocols, animals were water-deprived on the first three days of training, using a 23 hr fluid deprivation schedule to facilitate lever-pressing. Initially, the rats lever pressed for sucrose under a Fixed-Ratio 1 (FR1) schedule for about 5–7 days. Each lever press delivers 0.1 ml of reinforcer. During the second phase of the training, animals lever-pressed for an EtOH + sucrose cocktail mixture under a FR1 schedule. The concentration of sucrose was then decreased in a step-wise fashion (10%, 8%, 6%, 4%, 2% and 0%) and the EtOH was increased in a similar fashion (2%, 4%, 6%, 8% and 10%) over 5–7 days. Subsequently, the rats then responded under a FR4 schedule for EtOH (10% v/v) on both the right and left levers. The animals were allowed to continue to lever-pressing for EtOH (10% v/v) until their responses stabilized, defined as having daily responses within $\pm 20\%$ of the average responses for five consecutive days.

Surgery

The animals were anesthetized using isoflurane. After the animals were anesthetized and placed into the stereotaxic apparatus, a 22-gauge guide cannula was surgically implanted in the BST and contralaterally in the VTA or implanted in the NAcc and contralaterally in the VTA. The coordinates used for the BST were as follows: AP: -0.35 ; ML: $+2.5$; DV: -6.60 ; Degrees 8.7° from bregma. The coordinates for the NAcc were as follows: AP: $+1.60$; ML: $+1.80$; DV: -7.20 ; Degrees 8.5° from bregma. The coordinates used for the VTA were as follows: AP: -5.90 ; ML: $+1.60$; DV: -8.40 ; Degrees 6.0° from bregma. Cannulae were attached to the skull with anchor screws and dental cement. Each cannula was placed 1.0mm above the intended target. This allows for the 1.0mm that the injector tip extends below the cannula tip. The animals were allowed 7 days to recover before the continuation of the operant conditioning phase.

Infusion Procedure

The eticlopride and SR95531 were mixed prior to each infusion. The drug was mixed into 1 ml of sterile saline. The drug was then unilaterally infused into the target brain loci at a rate of $0.1\mu\text{l}/\text{min}$ for 5 minutes using a Harvard infusion pump. The injector tip was left in the cannula for an additional minute to facilitate the diffusion of all injected drug from the injector tip into

the brain loci. The animals were then placed into the operant chamber. After the rats received the initial infusion, subsequent infusions were not performed until bar-press responding for the reinforcers (i.e. EtOH or sucrose) returned to baseline (June et al., 2001; 2003; Foster et al., 2004).

Experimental Design

Eticlopride Infusions—The first phase of this study consisted of two separate groups of rats receiving unilateral infusions of eticlopride in the VTA alone. The first group consisted of animals implanted with cannula in the BST and VTA while the second group comprised rats implanted with a cannula in the NAcc and VTA. Both the BST/VTA group (N=8) and the NAcc/VTA group (N=6) were first trained to self administer EtOH (10% v/v) using the above mentioned EtOH self-administration procedure. The BST/VTA group then received 6 infusions of eticlopride (0.0, 2.5, 10.0, 20.0, 40.0, and 60.0 µg) into the VTA alone. These infusions were administered in a random order using a random number generator. The resulting dose order was as follows: 10.0 µg, 60.0 µg, 2.5 µg, 20.0 µg and 40.0 µg. The animals in the NAcc/VTA group also received 6 infusions of various doses of eticlopride (0.0, 2.5, 10.0, 20.0, 40.0, and 60.0 µg) into the VTA alone. All rats received all doses, and all doses were administered in a random fashion. The resulting dose order was as follows: 10.0 µg, 60.0 µg, 2.5 µg, 20.0 µg and 40.0 µg. The dose response employed was based on previous research in our laboratory demonstrating bilateral doses of eticlopride were effective in reducing EtOH responding in other mesolimbic loci (Foster et al., 2003 abstract).

SR 95531 Infusions—The second phase of the study consisted of the two groups of rats detailed above receiving unilateral infusions of SR 95531 in either the BST, or NAcc. The animals in the BST/VTA group received a single infusion of SR 95531 (32ng) into the BST while the animals of the NAcc/VTA group received the same dose of SR 95531 into the NAcc. The 32 ng dose was selected based on our preliminary data showing that it was the highest dose when given alone, which failed to modify intake of EtOH in the CeA (Foster et al., 2003). It was also selected because our preliminary data revealed that the 32ng dose was the lowest dose that completely reversed the effects of the D2 antagonist on EtOH intake following administration in other loci of the mesolimbic pathway (Foster et al., 2003).

Combination Infusions—In the third phase of this study, SR 95531 was given in combination with eticlopride in an attempt to block the effects of the D2 antagonist on EtOH self-administration. The animals in the BST/VTA group were microinfused with the 32ng dose of SR 95531 into the BST five minutes immediately prior to their receiving a 40µg dose of eticlopride into the VTA. Animals in the NAcc/VTA group received a similar combination dose with 32ng of SR 95531 being infused into the NAcc five minutes prior to a 40µg dose of eticlopride into the VTA. The 40µg dose of eticlopride was selected since our preliminary data revealed that this was the most effective unilateral dose which reduced EtOH intake in the VTA (Foster et al., 2003).

Histologies—After the completion of the behavioral component of the experiment, the animals were sacrificed using CO₂ inhalation. Prior to decapitation, cresyl violet was infused into the injection site at a rate 0.1 µl/min for 30 seconds to identify the placement of the cannulae. The brains were then immediately removed and frozen. Within 48 hours, the brains were sliced at 40 micron sections using a cryostat. The slides were then stained with cresyl violet and placement of the cannulae was verified using the Paxinos and Watson atlas (1998). The placements of the cannulae were independently determined by two individuals that are not aware of the behavioral data for the rats. Rats with misplacements were excluded from the statistical analysis.

Data Collection—The dependent variable for this study was the number of responses (lever presses) for reinforcer in a thirty minute operant paradigm session. For the mean number of responses analysis, the total number of responses in an entire thirty minute session was used. All data were collected using the L2T2, Coulbourn computer program (Coulbourn Instruments, Inc, Lehigh Valley, PA).

Data Analysis—All data were evaluated by the use of repeated-measures ANOVA's for mean number of responses and drug dose in relation to control conditions. The control condition (baseline) was defined by the combination of non-infusion control and saline infusion control for each group of subjects. In all groups, the result of the saline infusion was similar to the non-infusion control. This was followed by post hoc analysis of all ANOVA's by Duncan's test. All data analyses were preformed with the Statmost statistical package (Dataxiom Software Inc., Los Angeles, CA).

RESULTS

Histologies

Figures 1–2 show reconstructions of the serial coronal sections of the rat brain illustrating the location of the implanted microinjection cannulae. Figure 1 depicts the location of the unilaterally implanted BST microinjection cannulae and the contralaterally implanted unilateral VTA cannulae in the BST/VTA rat group. The cannula placements for the animals in the NAcc/BST group are depicted in Figure 2. Figure 3, panels A–B show examples of a representative photomicrograph for the two groups illustrating the extent of the lesions sustained as a result of the bilateral guide cannula implantation. Panel A depicts the BST tract from one representative animal from the BST/VTA group and the VTA tract from another representative animal from the BST/VTA group. Panel B of Figure 3 depicts the NAcc tract from a representative animal of the NAcc/VTA group is and the VTA tract from another representative animal from the NAcc/VTA group.

Neuroanatomical Specificity

Five animals were found to have cannula sites outside of the VTA. The cannula of four animals were found to be above the VTA in the vicinity of the red nucleus, while the cannula of the final animals was found to terminate below the VTA in the region of the interpeduncular nucleus. The data for these animals was analyzed to determine the neuroanatomical specificity of action by the tested compounds. The resulting analysis produced a nonsignificant one-way ANOVA [$F(5, 20) = 0.61, p > 0.05$]. This data suggests that the diffusion of the microinjected eticlopride is limited to the immediate area of the VTA surrounding the injection tip and that diffusion outside of the targeted substrate is unlikely. Unlike the VTA, the cannula aimed at the BST and NAcc were all correctly placed; however, previous studies conducted by this lab have examined the neuroanatomical specificity of drug action in these substrates. Most notably a 2003 study by Eiler et al. examined the neuroanatomical specificity of SCH 23390 in the BST by placing cannula in the caudate putamen (CPu) a substrate generally accepted as a viable neuroanatomical control for limbic structure. As seen in the figure below, SCH 23390 had no effect in the CPu while a robust effect was seen in the BST. While no data has been collected in the CPu with SR 95531, it can be easily theorized that SR 95531 would diffuse less than SCH 23390 due to its reduced solubility (5 mg/ml for SR 95531 vs. 8 mg/ml for SCH 23390). In short, previous research demonstrates that the diffusion of SR 95531 outside of the BST or NAcc is unlikely due to its relatively low solubility.

Eticlopride Infusions

BST/VTA Group—Figure 4 shows rate of responding maintained by EtOH following unilateral microinfusions of eticlopride (0.0 – 60.0 μg) into the VTA of the BST/VTA animals.

These data show that the 10.0 – 40.0 μ g doses of eticlopride produced clear dose-dependent reductions in EtOH self-administration. However, the 60.0 μ g dose was nearly identical to baseline levels. Compared with the control conditions (i.e., non-infusion and saline infusion), responding was reduced by 63–74%, yielding a significant main effect of drug treatment [$F(5, 35) = 5.13, p \leq 0.01$]. The Duncan post-hoc test confirmed that the 10.0 – 40.0 μ g doses significantly reduced EtOH-maintained responding ($p \leq 0.01$), while the 60.0 μ g dose was similar to control baseline levels ($p > 0.05$).

NAcc/VTA Group—The effects of the microinfusion of the D2 antagonist eticlopride into the VTA on EtOH self-administration in the NAcc/VTA rats are illustrated in Figure 5. As in the BST/VTA group above, only the moderate doses of eticlopride (10.0 – 40.0 μ g) reduced EtOH self-administration. When compared with the control condition, the 10.0, 20.0 and 40.0 μ g doses reduced responding by 41%, 63% and 94%, respectively. These data produced a significant main effect of drug treatment [$F(5, 25) = 11.03, p \leq 0.01$] with the 10.0 – 40.0 μ g doses significantly ($p \leq 0.01$) reducing EtOH self-administration as confirmed by Duncan's post-hoc testing. However, similar to the BST/VTA group, the 60.0 μ g dose was nearly identical to baseline levels ($p > 0.05$).

SR 95531 Infusions

BST/VTA Group—Illustrated in Figure 4 are the effects of a single microinfusion into the BST of the non-selective GABA antagonist, SR 95531 on EtOH self-administration. The 32ng dose of SR 95531 failed to alter EtOH responding when microinfused into the BST. These data yielded a non-significant main effect of drug treatment [$F(1, 7) = 0.11, p \geq 0.05$].

NAcc/VTA Group—Depicted within Figure 5 are the effects of SR 95531 microinjected into the NAcc on responding for 10% (v/v) EtOH. Unlike the BST/VTA group, the 32ng dose of SR 95531 infused into the NAcc did produce a significant main effect of treatment [$F(1, 5) = 11.14, p \leq 0.05$]. Post hoc testing revealed that the 32.0 ng dose of SR 95531 was sufficient to significantly ($p \leq 0.01$) reduce responding for EtOH (10% v/v).

Combination Infusions

BST/VTA Group—Figure 4 also depicts the effect of a 40 μ g dose of eticlopride into the VTA in combination with a 32ng dose of SR 95531 into the BST on EtOH reinforced responding. As noted above, given alone, the 40 μ g dose of eticlopride into the VTA led to a 74% reduction in EtOH self-administration; however, when the 32ng dose of SR 95531 was given into the BST immediately prior to the eticlopride, it completely reversed the eticlopride reduction on EtOH responding. When compared with the control conditions the combination SR 95531 + eticlopride condition was similar, resulting in a non-significant main effect of treatment condition [$F(1, 7) = 0.65, p \geq 0.05$]. However, the 40 μ g dose of eticlopride alone resulted in a significant treatment effect relative to the combination dose [$F(1, 7) = 26.97, p \leq 0.01$]. The effects of the combination treatment compared to eticlopride alone were confirmed by Duncan post hoc analyses ($p < 0.01$).

NAcc/VTA Group—Figure 5 depicts the effect of a 40 μ g dose of eticlopride into the VTA in combination with a 32ng dose of SR 95531 into the NAcc on EtOH reinforced responding. Given alone, the 40 μ g dose of eticlopride into the VTA led to a 94% reduction in EtOH self-administration; however, when the 32ng dose of SR 95531 was given into the NAcc immediately prior to the eticlopride, it reversed the eticlopride reduction on EtOH responding. Unlike the BST/VTA group, however, the SR 95531 did not reverse eticlopride's effect on responding completely. This is of little surprise as SR 95531 alone in the NAcc leads to a 46% reduction in EtOH-maintained responding. The combination dose did produce a significant main effect of drug treatment compared to the control condition [$F(1, 5) = 9.38, p \leq 0.05$] and

post hoc analysis confirmed that the combination effect was still well ($p < 0.01$) below control conditions. When the combination dose was compared to the 40 μ g dose of eticlopride alone, a significant main effect of drug treatment [$F(1, 5) = 14.42$, $p \leq 0.01$] emerged. Duncan's post-hoc analysis confirmed that the 32ng dose of SR 95531 into the NAcc significantly ($p < 0.01$) attenuated the effect of the 40 μ g dose of eticlopride into the VTA.

DISCUSSION

The results of the present study demonstrated that that unilateral microinjection of the selective D2 antagonist eticlopride into the VTA of P rats produced suppression of EtOH-maintained responding. However, a U-shaped dose response curve was observed in both the BST/VTA and the NAcc/VTA implanted animals, confirming the reliability of the eticlopride effect in the VTA. The present study also demonstrated that the unilateral microinfusion of the potent GABA_A receptor antagonist SR 95531 into the BST had no significant effect on EtOH self-administration at a 32.0 ng dose. In, contrast, the same dose unilaterally administered into the NAcc lead to a marked suppression of EtOH-maintained responding. When the 32.0 ng dose of SR 95531 was centrally administered in to either the BST or NAcc in combination with the most effective dose (40.0 μ g) of eticlopride, the GABA_A antagonist reversed the attenuation of the D2 antagonist in the VTA. As previously reported in outbred rats (Samson and Chappelle, 1999), these data elucidate a role for the D2 receptor system of the VTA in the mediation of EtOH reward in the P rat line, albeit they contrast the data of Nowak et al., (2000). The data of the present study further suggest that while the GABAergic systems within the BST may not have a direct effect on EtOH reinforcement, the GABA system of the extended amygdala may act as a modulator of DA via the VTA in regulating EtOH reinforcement

Effects of Eticlopride on EtOH Reward

Compelling evidence has been reported in the literature demonstrating that the D2 receptor subtype population within the VTA functions predominantly as somatodendritic autoreceptors (for review see Kalivas, 1993; Kohl et al., 1998). Blockade of these receptors by microinfusion of D2 antagonists directly into the VTA have consistently produced increases in extracellular DA release in the NAcc that mimics the increased release seen following EtOH administration (Kohl et al., 1998; Westerink et al., 1996). Taken together these findings suggest that any changes in EtOH self-administration following the infusion of a D2 antagonist into the VTA maybe due to changes in the regulatory DA autoreceptor system within the VTA. To test this hypothesis Nowak and colleagues (2000) evaluated the actions of the D2 antagonist, sulpiride on homecage EtOH drinking. They demonstrated that low doses (0.5–2.0 μ g) of sulpiride were ineffective in reducing EtOH intake in P rats. They hypothesized that the ineffectiveness of the D2 antagonist was due to an insufficient DA release resulting in a failure to alter EtOH intake. The present study paralleled the Nowak study in so far as the lowest dose (2.5 μ g) of eticlopride tested was similar to the highest tested dose in the Nowak study (2.0 μ g). As with the 2.0 μ g dose of sulpiride in the Nowak study, the 2.5 μ g dose of eticlopride failed to alter EtOH intake.

In contrast to the low D2 antagonist doses in the Nowak et al., (2000) and the present study, a 10.0 μ g dose of the D2 antagonist, raclopride produced a reduction in EtOH-maintained responding in the VTA of Long-Evans rats (Samson and Chappelle, 1999). This finding was further supported by the present study wherein a 10.0 μ g dose of eticlopride microinjected into the VTA of the both the BST/VTA and NAcc/VTA groups produced a significant reduction in operant responding for EtOH. The data from this study and Samson and Chappelle (1999) suggest that VTA-D2 reduction in EtOH intake may be due to increases in DA release within the NAcc, and possibly other limbic loci such as the BST. Hence, the D2-VTA reduction may be due to reward substitution as opposed to a decrease in the reward value of EtOH itself.

Moreover, the D2 antagonist, in high doses, may itself become reinforcing. Indeed, this has been suggested by several previous studies in the literature (see Parada et al., 1995; Kohl et al., 1998).

These data also produce an interesting effect in that the 60.0 µg dose of eticlopride failed to alter EtOH responding. This finding, though consistent in both implant groups, is more difficult to explain. As stated above, the 60.0 µg dose was the second drug treatment given ruling out possible order effects. Also, 24 post administration data was collected after each drug infusion with no effect on EtOH self-administration observed following the 60 µg dose adding additional veracity to the claim that the effects seen were not due to drug dose order effects. However, a possible explanation does emerge from literature suggesting that increases in extracellular DA within the NAcc can trigger a negative feed-back loop to the VTA that may in turn reduce dopamine release into the NAcc (Rahman and McBride, 2000). This feed back loop may be independent of the D2 autoreceptor system itself, relying possibly on amino acid or cholinergic input into the VTA (Rahman and McBride, 2000). If such a reduction were to occur, the rats may increase drinking back to baseline levels in an attempt to maintain DA levels within the NAcc. While such an explanation is merely conjecture without further study, the possibility that such a compensatory mechanism may regulate actions of the 60.0 µg eticlopride dose seem likely given the negative feed-back loop theory proposed by Rahman and McBride (2000).

Effects of SR 95531 on EtOH Reward

The present study also demonstrated that unilateral injections of the selective GABA_A antagonist, SR 95531, had no effect on EtOH-maintained responding when microinjected into the BST at a dose of 32.0 ng. This finding contrasts the work of Hyytiä and Koob (1995). Hyytiä and Koob found that doses of 8.0 and 16.0 ng of SR 95531 microinfused into the BST produced significant reductions in operant responding for EtOH (w/v). The 32.0 ng dose of SR 95531 was chosen for the present study since lower doses in our preliminary studies were ineffective in altering the injections of DA antagonists in other limbic loci. However, despite our efforts to compensate this difference with an increased drug dose, the use of unilateral as opposed to bilateral infusion of the GABA compound may explain the disparity in the findings. A closer examination of the data from the Hyytiä and Koob study may suggest another possibility. It appears that the 16.0 ng dose of SR 95531 into the BST appears to have less of an effect on EtOH responding than dose the 8.0 ng dose. This may suggest that SR 95531 may produce a U-shaped dose response curve with both lower and higher doses presenting as ineffectual on EtOH reinforcement, though the mechanism of action to explain this is unknown. Finally, it is possible the rodent model employed may explain the discrepant results. Little is known about the density of GABA_A receptors with the limbic loci in P rats, particularly the BST.

In contrast with the findings in the BST/VTA group, a unilateral injection of SR 95531 into the NAcc is sufficient to significantly reduce EtOH responding in the P rats. These data further confirm the findings presented by Hyytiä and Koob (1995) which demonstrated that a bilateral infusion of 16 ng of SR 95531 lead to a significant decrease in EtOH intake. While, our reduction in EtOH responding is not quite as robust as that seen by Hyytiä and Koob, this can easily be explained by the use of a unilateral microinjection in this study as opposed to bilateral delivery of the drug.

Effects of SR 95531 and Eticlopride in Combination on EtOH Reward

Data from the final portion of this study demonstrated that the suppression of EtOH-maintained responding produced by a 40.0 µg dose of eticlopride into the VTA can be attenuated by a 32.0 ng dose of SR 95531 into either the BST or the NAcc. This finding suggests the DAergic system within the mesolimbic pathway maybe directly or indirectly modulated by GABAergic

mechanisms. As stated above, both the NAcc and BST contain a large number of GABAergic neurons that interconnect all the components of the limbic system (Sun and Cassell, 1993), and there is also evidence that both the BST and NAcc send projections directly to the VTA (Georges and Aston-Jones, 2002; Kalivas et al., 1993). It is possible that the antagonism of GABAergic systems within these loci led to disinhibition of the dopaminergic system within these substrates, particularly the NAcc. Such disinhibition could potentially lead to increased DA release within the mesolimbic system that, though insufficient with SR 95531 alone, may interact with the increased DA levels produced by the 40.0 µg dose of eticlopride to activate a long feed back loop as purposed by Rahman and McBride (2000). Activation of this feed back loop could produce a decrease in accumbal DA release which the P rats may attempt to compensate for by increasing EtOH-maintained responding, thus returning response rates to near baseline levels. The present experiment design does not allow for a clear determination of a mechanism of action for the GABAergic attenuation of the D2 antagonist induced suppression of EtOH drinking; therefore, additional research is needed to further elucidate the exact neuromechanism of action by which GABA inhibition regulates the D2 reduction on alcohol-motivated responding.

Conclusions

The results of this study suggest that moderate doses (10.0 – 40.0 µg) of the D2 antagonist, eticlopride, unilaterally infused in the VTA are capable of significantly suppressing EtOH-maintained responding in P rats while low (2.5 µg) and high (60.0 µg) doses had no effect on EtOH reinforcement. This may indicate that the eticlopride itself may act as a reward, substituting itself for EtOH as the primary reinforcer. This study also determined that a 32.0 ng dose of the competitive GABA_A antagonist SR 95531 unilaterally administered into the BST was unable to alter EtOH responding while the same dose infused within the NAcc led to a reduction responding. However, the same dose of SR 95531 administered into either the BST or NAcc was sufficient to attenuate the suppression of EtOH responding seen with a 40.0 µg eticlopride dose within the VTA. These findings suggest a significant role of the D2 autoreceptor population within the VTA in the regulation of EtOH reinforcement; as well as demonstrating the ability of the GABAergic system within the BST and NAcc to mediate the VTA's effect on EtOH reward via the DA system.

Acknowledgments

This research was supported in part by grants AA10406, AA11555 (HLJ) from the National Institute of Alcohol Abuse and Alcoholism (NIAAA), and grants AA10422 and GM47818 (GEH) from National Institute of General Medical Science (NIGMS). The authors thank Drs. T.-K. Li, currently at NIAAA, and Larry Lumeng and the Alcohol Research Center, Indiana University School of Medicine for providing the P rats.

References

- Carboni E, Silvagni A, Rolando MT, Di Chiara G. Stimulation of in vivo dopamine transmission in the bed nucleus of stria terminalis by reinforcing drugs. *J Neurosci* 2000 Oct 15;20(20):RC102. [PubMed: 11027253]
- Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A* 1988;85(14):5274–8. [PubMed: 2899326]
- Eiler WJ 2nd, Seyoum R, Foster KL, Mailey C, June HL. D1 dopamine receptor regulates alcohol-motivated behaviors in the bed nucleus of the stria terminalis in alcohol-preferring (P) rats. *Synapse* 2003;48(1):45–56. [PubMed: 12557272]
- Foster KL, McKay PF, Seyoum R, Milbourne D, Yin W, Sarma PV, Cook JM, June HL. GABA(A) and opioid receptors of the central nucleus of the amygdala selectively regulate ethanol-maintained behaviors. *Neuropsychopharmacology* 2004;29(2):269–84. [PubMed: 14666116]

- Foster, KL.; Seyoum, R.; Goergen, J.; Mensah-Zoe, B.; June, HL. GABA/Dopamine Interaction in the Central Amygdala (CeA) Regulate Ethanol-Maintained Responding. Paper presented at the annual meeting of the Society for Neurosciences; Orlando, FL. November, 2003; 2003.
- Georges F, Aston-Jones G. Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. *J Neurosci* 2002;22(12):5173–87. [PubMed: 12077212]
- Gonzales RA, Job MO, Doyon WM. The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. *Pharmacol Ther* 2004;103(2):121–46. [PubMed: 15369680]
- Harvey SC, Foster KL, McKay PF, Carroll M, Seyoum R, Woods JE II, Grey C, McCane S, Cummings R, Mason D, Jones CM, Ma C, Cook JM, June HL. The GABA_A receptor $\alpha 1$ subtype in the ventral pallidum regulates EtOH-seeking behaviors. *Journal of Neuroscience* 2002;22:3766–3775.
- Hodge CW, Samson HH, Chappelle AM. Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. *Alcohol Clin Exp Res* 1997;21(6):1083–91. [PubMed: 9309321]
- Hodge CW, Chappelle AN, Samson HH. GABAergic transmission in the nucleus accumbens is involved in the termination of ethanol self-administration in rats. *Alcohol Clin Exp Res* 1995;19:1486–1493. [PubMed: 8749815]
- Hodge CW, Samson HH, Tolliver GA, Haraguchi M. Effects of intraaccumbens injections of dopamine agonists and antagonists on sucrose and sucrose-ethanol reinforced responding. *Pharmacol Biochem Behav* 1994;48(1):141–50. [PubMed: 7913224]
- Hodge CW, Haraguchi M, Erickson H, Samson HH. Ventral tegmental microinjections of quinpirole decrease ethanol and sucrose-reinforced responding. *Alcohol Clin Exp Res* 1993;17(2):370–5. [PubMed: 8098187]
- Hodge CW, Samson HH, Haraguchi M. Microinjections of dopamine agonists in the nucleus accumbens increase ethanol-reinforced responding. *Pharmacol Biochem Behav* 1992;43(1):249–54. [PubMed: 1357676]
- Hyytiä P, Koob GF. GABA_A receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. *Eur J Pharmacol* 1995;283:151–159. [PubMed: 7498304]
- Imperato A, Di Chiara G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J Pharmacol Exp Ther* 1986;239(1):219–28. [PubMed: 3761194]
- Jeziorski M, White FJ. Dopamine agonists at repeated “autoreceptor-selective” doses: effects upon the sensitivity of A10 dopamine autoreceptors. *Synapse* 1989;4(4):267–80. [PubMed: 2603146]
- June, HL.; Eiler, WJA, II. Dopaminergic and GABAergic Regulation of Alcohol-Motivated Behaviors: Novel Neuroanatomical Substrates. In: Sibley, David; Hanin, Israel; Kuhar, Michael; Skolnick, Phil, editors. *The Handbook of Contemporary Neuropsychopharmacology*. John Wiley and Sons; NY, NY: in press
- June HL, Foster KL, McKay PF, Seyoum R, Woods JE, Harvey SC, Eiler WJ, Grey C, Carroll MR, McCane S, Jones CM, Yin W, Mason D, Cummings R, Garcia M, Ma C, Sarma PV, Cook JM, Skolnick P. The reinforcing properties of alcohol are mediated by GABA(A1) receptors in the ventral pallidum. *Neuropsychopharmacology* 2003;28(12):2124–37. [PubMed: 12968126]
- June HL, Harvey SC, Foster KL, Mc Kay PF, Cummings R, Garcia M, Mason D, Grey C, Mc Cane S, Williams L, Johnson TB, He X, Rock S, Cook J. GABA_A receptors containing $\alpha 5$ subunits in the CA1 and CA3 hippocampal fields regulate ethanol-motivated behaviors: an extended ethanol reward circuitry. *J Neurosci* 2001;21:2166–2177. [PubMed: 11245701]
- June HL, Grey C, Warren-Reese C, Durr L, Ricks-Cord, Johnson A, McCane A, Williams S, Mason DL, Cummings R, Lawrence A. The opioid receptor antagonist nalmefene reduces responding maintained by ethanol presentation: preclinical studies in Ethanol-Preferring and outbred wistar rats. *Alcohol Clin Exp Res* 1998a;22:2174–2185. [PubMed: 9884166]
- June HL, Torres L, Cason CR, Hwang BH, Braun MR, Murphy JM. The novel benzodiazepine inverse agonist RO19-4603 antagonizes ethanol motivated behaviors: neuropharmacological studies. *Brain Res* 1998b;784(1–2):256–75. [PubMed: 9518641]
- June HL, Hughes RW, Spurlock HL, Lewis MJ. Ethanol self-administration in freely feeding and drinking rats: effects of Ro15-4513 alone, and in combination with Ro15-1788 (flumazenil). *Psychopharmacology (Berl)* 1994a;115(3):332–9. [PubMed: 7871073]

- June HL, Murphy JM, Mellor-Burke JJ, Lumeng L, Li TK. The benzodiazepine inverse agonist RO19-4603 exerts prolonged and selective suppression of ethanol intake in alcohol-preferring (P) rats. *Psychopharmacology (Berl)* 1994b;115(3):325–31. [PubMed: 7871072]
- Kalivas PW. Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res Brain Res Rev* 1993;18(1):75–113. [PubMed: 8096779]
- Kalivas PW, Churchill L, Klitenick MA. GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. *Neuroscience* 1993;57(4):1047–60. [PubMed: 7508582]
- Kohl RR, Katner JS, Chernet E, McBride WJ. Ethanol and negative feedback regulation of mesolimbic dopamine release in rats. *Psychopharmacology (Berl)* 1998;139(1–2):79–85. [PubMed: 9768545]
- McBride WJ, Li TK. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol* 1998;12(4):339–69. [PubMed: 10348615]
- McBride WJ, Murphy JM, Lumeng L, Li TK. Effects of Ro 15-4513, fluoxetine and desipramine on the intake of ethanol, water and food by the alcohol-preferring (P) and -nonpreferring (NP) lines of rats. *Pharmacol Biochem Behav* 1988;30(4):1045–50. [PubMed: 3265788]
- Melendez RI, Rodd ZA, McBride WJ, Murphy JM. Involvement of the mesopallidal dopamine system in ethanol reinforcement. *Alcohol* 2004;32(2):137–44. [PubMed: 15163564]
- Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Lumeng L, Li TK. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behav Genet* 2002;32(5):363–88. [PubMed: 12405517]
- Nowak KL, McBride WJ, Lumeng L, Li TK, Murphy JM. Involvement of dopamine D2 autoreceptors in the ventral tegmental area on alcohol and saccharin intake of the alcohol-preferring P rat. *Alcohol Clin Exp Res* 2000;24:476–83. [PubMed: 10798583]
- Oades RD, Halliday GM. Ventral tegmental (A10) system: neurobiology. I. Anatomy and connectivity. *Brain Res Rev* 1987;12:117–165.
- Parada MA, Puig de Parada M, Hoebel BG. Rats self-inject a dopamine antagonist in the lateral hypothalamus where it acts to increase extracellular dopamine in the nucleus accumbens. *Pharmacol Biochem Behav* 1995 Sep;52(1):179–87. [PubMed: 7501663]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Sydney: Academic Press; 1998.
- Rahman S, McBride WJ. Feedback control of mesolimbic somatodendritic dopamine release in rat brain. *J Neurochem* 2000;74(2):684–92. [PubMed: 10646520]
- Rassnick S, D'Amico E, Riley E, Koob GF. GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. *Alcohol Clin Exp Res* 1993;17(1):124–30. [PubMed: 8383923]
- Rassnick S, Pulvirenti L, Koob GF. Oral ethanol self-administration in rats is reduced by the administration of dopamine and glutamate receptor antagonists into the nucleus accumbens. *Psychopharmacology (Berl)* 1992;109(1–2):92–8. [PubMed: 1365677]
- Samson HH, Chappelle AM. Effects of microinjection of the D2 dopamine antagonist raclopride into the ventral tegmental area on ethanol and sucrose self-administration. *Alcohol Clin Exp Res* 1999;23(3):421–6. [PubMed: 10195813]
- Samson HH, Hodge CW, Tolliver GA, Haraguchi M. Effect of dopamine agonists and antagonists on ethanol-reinforced behavior: the involvement of the nucleus accumbens. *Brain Res Bull* 1993;30(1–2):133–41. [PubMed: 8093596]
- Samson HH, Tolliver GA, Haraguchi M, Hodge CW. Alcohol self-administration: role of mesolimbic dopamine. *Ann N Y Acad Sci* 1992;654:242–53. [PubMed: 1352952]
- Samson HH, Tolliver GA, Pfeffer AO, Sadeghi KG, Mills FG. Oral ethanol reinforcement in the rat: effect of the partial inverse benzodiazepine agonist RO15-4513. *Pharmacol Biochem Behav* 1987;27(3):517–9. [PubMed: 3659074]
- Sun N, Cassell MD. Intrinsic GABAergic neurons in the rat central extended amygdala. *J Comp Neuro* 1993;330:381–404.
- Wegelius K, Honkanen A, Korpi ER. Benzodiazepine receptor ligands modulate ethanol drinking in alcohol-preferring rats. *Eur J Pharmacol* 1994;263(1–2):141–7. [PubMed: 7821345]

- Westerink BH, Kwint HF, de Vries JB. The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. *J Neurosci* 1996 Apr 15;16(8):2605–11. [PubMed: 8786436]
- Yoshimoto K, McBride WJ, Lumeng L, Li TK. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. *Alcohol* 1992;9(1):17–22. [PubMed: 1370758]

Figure 1. Reconstruction of serial coronal sections of the alcohol-preferring (P) rat brains illustrating the unilateral (N=8) guide cannula tips in the bed nucleus of the stria terminalis (BST) as well as the unilateral guide cannula tips implanted contralaterally in the ventral tegmental area (VTA). Coronal sections are adapted from the rat brain atlas of Paxinos and Watson (1998).

Figure 2.

Reconstruction of serial coronal sections of the alcohol-preferring (P) rat brains illustrating the unilateral (N=6) guide cannula tips in the nucleus accumbens (NAcc) as well as the unilateral guide cannula tips implanted contralaterally in the ventral tegmental area (VTA). Each rat is represented by one solid black circle in the left and one in the right hemisphere. Coronal sections are adapted from the rat brain atlas of Paxinos and Watson (1998).

Figure 3.

[A] Representative histological photomicrographs of the actual unilateral guide cannula placements in the bed nucleus of the stria terminalis (BST) for one representative alcohol-preferring (P) rat and the actual unilateral guide cannula placements in the ventral tegmental area (VTA) for another representative alcohol-preferring (P) rat [B] and actual unilateral guide cannula placements in the nucleus accumbens (NAcc) for one representative alcohol-preferring (P) rat and the actual unilateral guide cannula placements in the ventral tegmental area (VTA) for another representative alcohol-preferring (P) rat following the infusion of cresyl violet.

Figure 4.

Effects of eticlopride (2.5 – 60.0 μg) following unilateral injection in the ventral tegmental area (VTA) on responding maintained by EtOH (10% v/v). Also depicted are the effects of SR 95531 (32.0 ng) following unilateral injection in the bed nucleus of the stria terminalis (BST) on responding maintained by EtOH (10% v/v). The effects of the combination dose of eticlopride (40.0 μg) unilaterally injected into the VTA along with SR 95531 (32.0 ng) unilaterally injected in the BST on responding maintained by EtOH (10% v/v). ** $p \leq 0.01$ eticlopride vs. baseline (non-infusion and saline infusion controls). †† $p \leq 0.01$ combination dose vs. 40.0 μg dose of eticlopride alone. Data are shown as mean ($\pm$ S.E.M.).

Figure 5.

Effects of eticlopride (2.5 – 60.0 μ g) following unilateral injection in the ventral tegmental area (VTA) on responding maintained by EtOH (10% v/v). Also depicted are the effects of SR 95531 (32.0 ng) following unilateral injection in the nucleus accumbens (NAcc) on responding maintained by EtOH (10% v/v). The effects of the combination dose of eticlopride (40.0 μ g) unilaterally injected into the VTA along with SR 95531 (32.0 ng) unilaterally injected in the NAcc on responding maintained by EtOH (10% v/v). ** $p \leq 0.01$ eticlopride vs. baseline (non-infusion and saline infusion controls). †† $p \leq 0.01$ combination dose vs. 40.0 μ g dose of eticlopride alone. Data are shown as mean ($\pm$ S.E.M.).