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Research report

$GABA_A$ receptors in the posterior, but not anterior, ventral tegmental area mediate Ro15-4513-induced attenuation of binge-like ethanol consumption in C57BL/6J female mice

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ABSTRACT

$GABA_A$ receptors have been shown to modulate dopaminergic output from the ventral tegmental area (VTA) in studies of both natural and drug rewards, including alcohol. Ro15-4513, the imidazobenzodiazepine derivative and allosteric modulator at the $GABA_A$ receptor, reliably antagonizes the behavioral effects of alcohol. Various models of alcohol consumption show a decrease in consummatory behaviors, specific to ethanol, following acute administration of the drug. In the present study, Ro15-4513 was systemically administered, or microinjected into the anterior or posterior VTA, to explore the role of $GABA_A$ receptors at this region in modulating the high pattern of alcohol consumption by C57BL/6J inbred mice in the Drinking in the Dark (DID) model. Animals had 2 h access to ethanol for 6 days prior to drug manipulations. Immediately before the seventh day of access, mice were systemically (I.P.) or site-specifically administered Ro15-4513. Systemic Ro15-4513 (at 10 mg/kg) decreased binge-like ethanol intake in the DID paradigm. Additionally, there was a stepwise decrease in consumption following Ro15-4513 microinjection into the posterior VTA, with the highest dose significantly decreasing ethanol intake. There was no effect found following microinjection into the anterior VTA, nor was there an effect of systemic or intra-posterior VTA Ro15-4513 on consumption of a 5% sucrose solution or water. The present findings support a role for Ro15-4513 sensitive VTA-$GABA_A$ receptors in modulating binge-like ethanol consumption. Moreover, the work here adds to the growing body of literature suggesting regional heterogeneity in the VTA.

1. Introduction

Binge alcohol consumption is an important behavioral marker of many alcohol use disorders. Pharmacologically defined as alcohol intake producing a blood alcohol concentration (BAC) of 80 mg/dL or greater [1], this level of drinking results in behavioral intoxication and presumably activates the neurobiological systems involved in the reinforcing properties of alcohol. Specifically, given the supported role of receptor systems localized to the mesocorticolimbic pathway in regulating reinforcement [2–6], modulation of said pathways should alter patterns of binge drinking. Due to ethical constraints, animal models have been vital in our efforts to explore the role of various receptor systems in mediating ethanol related behaviors. In particular, the Drinking in the Dark (DID) limited access paradigm allows us to model binge-ethanol intake in various inbred mouse strains [7,8]. We have recently begun to use this DID procedure to investigate the role of receptor systems in the ventral tegmental area (VTA), that modulate activity along the mesocorticolimbic reward pathway, in mediating binge intake of ethanol.

The VTA contains GABAergic neurons that both locally and distally inhibit activity in the reward pathway [9,10]. $GABA_A$ receptors, in particular, have been shown to mediate tonic inhibition of this dopaminergic pathway [11–13]. For example, David et al. [14] showed that mice would successfully self-administer bicuculline ($GABA_A$ antagonist) into the VTA and that this self-administration could be extinguished by pretreatment with sulpiride, a dopamine receptor antagonist.

$GABA_A$ receptors are interesting pharmacological targets because of their structural diversity. This pentameric ionophore complex may be composed of multiple protein classes, many of which exist as varying isoforms. The subunit composition of the $GABA_A$ receptor complex confers unique pharmacological properties to the receptor subtypes [15]. For example, various α subunit

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isoforms have been associated with benzodiazepine insensitivity. Interestingly, it is posited that Ro15-4513, a compound repeatedly shown to antagonize the behavioral and physiological effects of alcohol, may be exerting its effects by competing with alcohol for respective binding sites on these benzodiazepine-insensitive receptors [16,17].

Ro15-4513 has long been considered an antagonist of alcohol's actions at the $GABA_A$ receptor complex. Electrophysiological evidence in synaptoneurosome preparations [18,19] support an attenuation of ethanol's potentiation of $GABA_A$ mediated chloride flux, following Ro15-4513 application. Behaviorally, the drug has been shown to block the sedative-hypnotic, anxiolytic and intoxicating effects of ethanol in various rodent models [20–25]. Ro15-4513 has also successfully attenuated self-administration of ethanol in various paradigms [26–28]. The consistent attenuation of alcohol responding induced by Ro15-4513 pretreatment has been interpreted as a reduction in the motivational properties of the drug [29]. However, experimental evidence does not necessarily support this interpretation. For example, Risinger et al. [30] found that Ro15-4513 did not alter the induction of a conditioned response to an environment paired with ethanol administration. Site-specific administration of the drug, to reward relevant areas like the VTA, may clarify its mechanism for reducing ethanol responding.

Significant evidence to date suggests anatomical and functional diversity in the VTA regarding its role in mediating reinforcement. Anatomically, the anterior and posterior regions of the VTA have distinct projections (most efferents to the medial prefrontal cortex from the VTA, for example, originate from the posterior zones; [31]) as well as varied levels of innervations at other terminal sites [32]. This heterogeneity has been shown to extend to functional responses, especially in terms of the GABAergic system and its role in mediating the effects of ethanol. Work from our laboratory found bidirectional effects of modulating the metabotropic GABA receptor system on ethanol-induced locomotor stimulation [33]. In this study, anterior VTA administration of the $GABA_B$ agonist baclofen, decreased ethanol-induced hyperlocomotion; the opposite effect was found following posterior-VTA administered baclofen. This functional heterogeneity, importantly, extended to manipulation of binge-like ethanol intake in the Drinking in the Dark model [34]. This diversity has been demonstrated for other receptor systems [35,36].

The ionotropic GABA receptor complex has also been shown to have a unique relationship with the anterior and posterior VTA regions. Ikemoto et al. [37] showed that rats in an operant self administration paradigm would only self-administer $GABA_A$ antagonists (like picrotoxin) into the anterior VTA. Furthermore, the same laboratory was able to establish that rats in the same paradigm would only self-administer $GABA_A$ agonists (like muscimol and ethanol) into the posterior VTA [38,39]. The possibility remains that the heterogeneity of VTA responsivity to $GABA_A$ ligands is also related to differences in $GABA_A$ receptor subtype across the anterior and posterior zones of the structure.

The present study sought to investigate the role of the "alcohol antagonist" Ro15-4513 in mediating binge-like intake in the alcohol preferring C57BL/6J inbred mouse strain, using the DID paradigm. Further, we wished to explore the role of the anterior and posterior VTA in mediating the effects of this drug on binge intake. Given the previously discussed effects of Ro15-4513 on operant self-administration of ethanol, we hypothesize that Ro15-4513 will decrease binge like ethanol intake following systemic administration. In addition, given the findings from our lab and others described earlier, we hypothesize that site specific administration of Ro15-4513 will produce bidirectional effects on binge-like ethanol intake. Specifically, we predict an increase in binge consumption following posterior-VTA administration and a decrease in binge consumption following anterior-VTA infusion of Ro15-4513.

2. Materials and methods

2.1. Animals

Subjects were female C57BL/6J inbred mice bred and maintained at Binghamton University (Experiment 1a and 1b) or Indiana University-Purdue University Indianapolis (IUPUI) School of Science (Experiment 1c and Experiment 2a through 2c). Mice were 70–100 days old at the start of each experiment. Animals were individually housed in standard shoebox cages and habituated to a 12 h reverse light/dark schedule for at least 7 days. The temperature of the colony rooms was maintained at $21 \pm 1\,^{\circ}C$. Food was available ad libitum except during stereotaxic surgery. Water was available ad libitum except during stereotaxic surgery (for all animals in Experiment 2) and when ethanol was made available as per DID protocol (see below). Procedures for Experiments 1a and 1b were approved by the Binghamton University Institutional Animal Care and Use Committee (IACUC). Procedures for Experiments 1c and all of Experiment 2 were approved by the IUPUI School of Science IACUC. Procedures in all experiments conformed to NIH Guidelines for the care and use of mammals in Neuroscience and Behavioral Research [40].

2.2. Drugs and drinking solutions

Ethanol (190 proof) was obtained from Pharmco, Inc (Brookfield, CT). Ethanol drinking solutions (20% v/v) were made with tap water. Sucrose drinking solution (5% w/v) was prepared by dissolving sucrose (Sigma–Aldrich, St. Louis, MO) in tap water. Ro15-4513 15-4513 was obtained from Sigma–Aldrich (St. Louis, MO). The drug and final drug solutions were stored in opaque plastic bottles or foil wrapped glass vials. For systemic administration, the drug was suspended in Tween-80 and diluted with sterile physiological saline. Vehicle administered in systemic experiments consisted of physiological saline and Tween-80. For microinjections, the drug was solubilized using dimethylsulfoxide (DMSO, Sigma–Aldrich, St. Louis, MO) and serially diluted to final concentrations. This process reduced the final concentration of DMSO in the drug solutions to 0.01–0.1%. Vehicle administered in the microinjection experiment was a solution of 0.1% DMSO in physiological saline.

2.3. Drinking in the Dark

Beginning 3 h following lights out, water bottles were removed from each animal and replaced with modified sipper tubes made from 10 mL graduated cylinders fitted with double ball bearing sippers (Ancare, Belmore, NY). Modified drinking tubes contained ethanol (Experiment 1a and 2a), water (Experiment 1b and 2b) or a 5% (w/v) sucrose solution (Experiment 1c and 2c). Animals had access to drinking tubes for 2 h; there was no access to regular water bottles during this limited access procedure. Fluid intake was recorded as the change in fluid level along the modified drinking tube graduations.

2.4. Microneurosurgery

Guide cannulae, stylets and tubing to make injection cannulae were obtained from Small Parts Inc. (Miami Lakes, FL). Guide cannulae were made of 25-gauge stainless steel tubing, pre-cut to 15.5 mm. Stainless steel wire (0.0095 in.) was used to make stylets, which were inserted inside the guide cannulae to prevent obstruction. The guide cannulae were implanted 3 mm above the VTA by stereotaxic surgery (Model 1900; David Kopf Instruments, Tujunga, CA) as previously described [33,34]. Mice were first anesthetized using a ketamine/xylazine cocktail (containing 100 mg of ketamine and 10 mg of xylazine in 10 mL saline), administered intraperitoneally in a volume of 0.1 mL/10 g of mouse weight. The animal's dorsal scalp was shaved and a midline incision was made, revealing the skull from bregma to lambda (about 3 mm wide). The skull was cleaned with surgical scrub and sterilized using 100% ethanol (Pharmco Inc, Brookfield, CT). The 1997 edition of Franklin and Paxinos' *Mouse Brain Atlas* was used for coordinates. The anterior-VTA coordinates (from bregma: caudal 3.16 mm, lateral 0.5 mm, and ventral 2.0 mm) and posterior-VTA coordinates (from bregma: caudal 3.64 mm, lateral 0.5 mm, and ventral 2.0 mm) were adjusted for each mouse. To accomplish this, the published distance between bregma and lambda in this species (4.21 mm) was divided by the distance between bregma and lambda measured for each individual mouse. This quotient was then multiplied to each anterior or posterior coordinate listed above. Bilateral craniotomy holes were drilled at these individualized coordinates for placement of the guide cannulae. A third hole was drilled for an anchor screw (this third hole was enlarged using an 1/8 in. hand-held drill; Small Parts, Miami Lakes, FL, USA). Guide cannulae were lowered into position (2 mm above VTA region of interest) and durelon carboxylate cement (Norristown, PA, USA) was applied to the exposed cranium to hold the assembly in place. Animals remained in the stereotaxic apparatus until cement dried. Following surgery, mice were subcutaneously administered the anti-inflammatory drug carprofen (Rimadyl, 5 mg/kg, Pfizer Animal Health, USA), and the analgesic drug buprenorphine (Buprenex, 0.06 mg/kg, Reckitt and Coleman Pharmaceuticals, Richmond, VA) and were monitored for healthy recovery.