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Behavioural Brain Research 171 (2006) 207–215
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

The metabotropic glutamate 2/3 receptor agonist LY404039 reduces alcohol-seeking but not alcohol self-administration in alcohol-preferring (P) rats

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Received 17 October 2005; received in revised form 21 March 2006; accepted 27 March 2006
Available online 6 May 2006

Abstract

Metabotropic glutamate (mGlu) receptors have been shown to mediate a number of behaviors including emotionality and responsivity to stress as demonstrated by efficacy in preclinical and clinical studies. The objective of this study was to assess the effects of the mGlu2/3 receptor agonist LY404039 (LY) on operant ethanol (EtOH) self-administration during alcohol seeking (pavlovian spontaneous recovery, PSR), alcohol relapse (alcohol deprivation effect, ADE), and maintenance responding for alcohol. Adult alcohol-preferring (P) rats were trained in 2-lever operant chambers to self-administer 15% EtOH (v/v) and water on a concurrent fixed-ratio 5–fixed-ratio 1 (FR5–FR1) schedule of reinforcement in daily 1 h sessions. After at least 10 weeks of daily 1 h sessions, rats underwent seven extinction sessions, followed by 2 weeks of no manipulation, and then rats were tested for the expression of an EtOH PSR for four sessions. Rats were then given a week in their home cage before being returned to the operant chambers with access to EtOH and water (alcohol relapse). Finally, the effects of LY upon maintenance EtOH and water responding were assessed once stable responding was reestablished. The mGlu2/3 receptor agonist LY404039 reduced responding on the EtOH in the PSR test. LY also reduced the expression of an alcohol deprivation effect (ADE) during relapse, but did not reduce EtOH responding under maintenance conditions. The results of this study demonstrate that activating mGlu2/3 receptors inhibits the expression of alcohol seeking and relapse behavior without altering alcohol self-administration behavior.

Keywords: Ethanol self-administration; Ethanol relapse; Pavlovian spontaneous recovery; LY404039; Metabotropic glutamate receptor agonist; Alcohol-seeking

Group II metabotropic glutamate (mGlu) receptors are G-protein coupled receptors that modulate glutamate and non-glutamate neuronal activity through presynaptic, postsynaptic, and glial pathways. The mGlu2 receptors are located presynaptically and function as a negative feedback system to inhibit the release of glutamate [1,2]. The mGlu3 receptors are primarily located postsynaptically on neurons and glia [1]. Combined mGlu2/3 receptor agonists reduce the excitatory effect of glutamate postsynaptically [3]. However, mGlu2/3 receptor agonists also suppress electrically evoked and serotonin-induced postsynaptic activation through a presynaptic mechanism [4].

The mGlu2/3 receptor system is widely distributed throughout the frontal cortex and limbic system and is known to mediate a number of glutamate sensitive behaviors. At concentrations that do not impair normal functioning in animals, mGlu2/3 receptor agonists exhibit an anxiolytic profile in multiple animal models [5–8]. Moreover, clinical efficacy has been demonstrated with the mGlu2/3 agonist LY354740 in fear-potentiated startle [9] and CO2-induced panic provocation [10] paradigms. In withdrawal models of drug dependence, mGlu2/3 agonists attenuate withdrawal symptoms in nicotine- and morphine-dependent rats [11–13]. As elevations in glutamatergic tone have been observed to occur in response to both external and internal (e.g., drug withdrawal) stressors [14–17], it is postulated that mechanisms that reduce glutamate efflux may exhibit efficacy under such conditions of hyperglutamatergic tone. Indeed, one

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doi:10.1016/j.bbr.2006.03.032

of the pharmacological effects of acamprosate is to reduce elevated glutamate levels resulting from ethanol withdrawal [18,19].

The mGlu2/3 receptor system has been shown to be involved in various actions of drugs of abuse and to mediate drug-seeking/craving behaviors. The mGlu2/3 receptor agonist LY379268 was shown to reduce the expression of amphetamine conditioned locomotor sensitization [67], and either acute or sensitized phencyclidine (PCP)-induced locomotor activity [20]. More recently, the mGlu2/3 agonist LY379268 has demonstrated robust efficacy in cue-induced reinstatement of responding for both cocaine [21] and heroin [22].

Prior to the late 1980s, the majority of researchers considered extinction, the loss of instrumentally learned behavioral responses or the loss of a Pavlovian conditioned response, as 'unlearning' or the destruction of old learning/memory (e.g., [23,24]). Although this hypothesis may be intuitive, the past 20 years of research have indicated that extinction is not unlearning [25–32]. The current view of extinction is that it is not a process to destroy first-learned information, but is a process of obtaining new learning [27,28,32,31]. The results of numerous studies have indicated that following extinction training both the first-learned and second-learned signals are present, and that the signal or action is ambiguous (cf. [27,28,31]). The ambiguous quality of the signal/action following extinction is defined by the context.

There are at least four learning phenomena indicating that extinction does not produce unlearning (cf. [27]). The most common example of the retention of first-learned signals is the drug-reinstatement of responding model. US-induced reinstatement (recovery of a behavior that occurs following extinction training when the animal is non-contingently administered the US) has been used to examine seeking-behaviors for various drugs of abuse (cf. [33,34]). Extinction training in a novel context results in the renewal phenomenon (a recovery of extinguished behavior(s) when reintroduced into the original context). Reacquisition (the fast, often rapid, recovery of responding when the CS is paired with the US again) indicates that the first-learned signals are maintained following extinction. Additionally, reacquisition testing has shown the presence of both first- and second-learned signals. In multiple experiments the existence of second-learned signals is indicated since discriminative stimuli paired with extinction training can reduce the rate of reacquisition [35–37]. Extinction learning (second-learned) persists for an extremely long time since cues associated with extinction training can alter reacquired operant responding for months following extinction [69]. The last phenomenon to indicate that extinction does not produce unlearning is spontaneous recovery (pavlovian spontaneous recovery: PSR).

PSR is defined as a recovery of responding, in the absence of the previously trained reward, which is observed following a period of rest after extinction [38,39]. Conceptually, PSR is a unique phenomenon in that it is time dependent, and the behavior appears to be dependent on the re-exposure of the organism to all the cues in the behavioral environment previously associated with the reinforcer. While this may intuitively lead to the assumption that PSR responding is the product of 'forgetting about extinction', research has indicated that this is not the case. The majority of researchers hold that PSR occurs because there is a temporal context to extinction training. Specifically, as time increases between extinction training there is a shift in the ambiguity framework between first- and second-learned signals [25,40,41]. This stance is supported by research which has shown that retrieval cues for both first- and second-learned signals can alter PSR responding [42–44]. Thus, the PSR phenomenon can be viewed as a shift from expressing second-learned signals to first-learned signals without an elimination of either learning. The expression of a PSR is directly correlated to reward saliency [39,45], contextual cues associated with first-learned signals, and the amount of first- and second-learned associations [42]. In general, the PSR phenomenon has been asserted to be the result of a intrinsic shift away from the recent extinction (second-) learning to the initial reinforced learning responses, which reflects an intrinsic motivation to obtain the previously administered reward [27,28,31]. Therefore, the PSR model may represent a unique paradigm to study craving-like behaviors.

Studies assessing ethanol-seeking behaviors through expression of a PSR have been recently conducted in the alcohol-preferring (P) rat. First, P rats readily express a PSR for EtOH under operant conditions [46,47]. Peri-adolescent EtOH drinking potentiates the expression of a EtOH PSR when tested during adulthood [46]. Additionally, the expression of a EtOH PSR can be enhanced by exposure to EtOH odor or EtOH priming [46,47]. Thus, responding in the PSR test has a high degree of face validity for an animal model of alcohol-seeking behavior (cf. [48]).

The objective of the current experiment was to examine, using P rats, the effects of the mGlu2/3 receptor agonist LY404039 (LY) on operant ethanol (EtOH) responding under maintenance and ethanol-seeking conditions. LY is a potent and selective Group II metabotropic receptor agonist. Radioligand binding studies using LY to displace the mGlu2/3 antagonist ^{3}H-LY341495 reveal K_i values of 88 nM in rat forebrain tissue. Functional cAMP assays in cloned human mGlu receptors determine EC_{50} values of 23 and 48 nM for mGlu2 and mGlu3, respectively (unpublished data). No appreciable binding affinities were observed for other mGlu or iGlu receptor subtypes or glutamate transporters. These data indicate that LY404039 has a comparable in vitro binding profile to that of LY354740 [49]. In addition, the effects of LY were assessed on ethanol self-administration under relapse and maintenance conditions. EtOH self-administration under relapse conditions was assessed through expression of an alcohol deprivation effect (ADE), which is defined as a transient increase in voluntary EtOH intake following a period of ethanol deprivation [50–54,70]. Also, experiments were conducted with saccharin under PSR and relapse conditions in order to evaluate the effects of the LY compound on another reinforcer. The overall hypothesis to be tested is that LY would reduce EtOH-seeking behavior at doses that did not alter EtOH self-administration.