Corticotropin-Releasing Factor 1 Antagonists Selectively Reduce Ethanol Self-Administration in Ethanol-Dependent Rats

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Background: *Alcohol dependence is characterized by excessive alcohol consumption, loss of control over intake, and the presence of a withdrawal syndrome, which includes both motivational and physical symptoms. Similar to human alcoholics, ethanol-dependent animals display enhanced anxiety-like behaviors and enhanced ethanol self-administration during withdrawal, effects hypothesized to result from a dysregulation of corticotropin-releasing factor (CRF) stress systems. Here, we used an animal model of ethanol dependence to test the effects of CRF_1 receptor antagonists on excessive ethanol self-administration in dependent rats.*

Methods: *Wistar rats, trained to orally self-administer ethanol, were exposed intermittently to ethanol vapors to induce ethanol dependence. Nondependent animals were exposed to control air. Following a 2-hour period of withdrawal, dependent and nondependent animals were systemically administered antalarmin, MJL-1-109-2, or R121919 (CRF_1 antagonists) and ethanol self-administration was measured.*

Results: *The nonpeptide, small molecule CRF_1 antagonists selectively reduced excessive self-administration of ethanol in dependent animals during acute withdrawal. The antagonists had no effect on ethanol self-administration in nondependent rats.*

Conclusions: *These data demonstrate that CRF_1 receptors play an important role in mediating excessive ethanol self-administration in dependent rats, with no effect in nondependent rats. CRF_1 antagonists may be exciting new pharmacotherapeutic targets for the treatment of alcoholism in humans.*

Key Words: Alcohol, dependence, withdrawal, corticotropin-releasing factor, self-administration, rat

Alcoholism, defined as a chronic relapsing disorder characterized by compulsive use of alcohol and a loss of control over intake, is one of the leading causes of premature death in America (Stinson et al 1993). As dependence develops, there is a shift from controlled use to uncontrolled, excessive consumption of alcohol, which is paralleled by a shift from positive to negative reinforcement being the driving force mediating continued alcohol use (Koob 2003; Koob et al 2004). Initial alcohol use is driven mainly by the positive effects of alcohol, such as euphoria and tension reduction. However, with chronic alcohol consumption, cessation of use is often accompanied by negative withdrawal symptoms, such as increased anxiety and depression. Alleviation of these negative affect states (i.e., negative reinforcement) then becomes a major driving force for continued alcohol consumption (Hershon 1977; Koob 2003). Similar to human alcoholics, ethanol-dependent animals display enhanced anxiety-like behaviors and excessive ethanol self-administration during periods of withdrawal (Baldwin et al 1991; File et al 1989; O'Dell et al 2004; Roberts et al 2000; Valdez et al 2002b), providing a model system for studying the motivational changes associated with ethanol dependence. A better understanding of the neurobiological basis underlying ethanol reinforcement will be valuable for understanding the progression of ethanol dependence and developing novel pharmacotherapies for treatment.

Endogenous brain corticotropin-releasing factor (CRF) has been implicated in the motivational changes associated with ethanol dependence (Menzaghi et al 1994; Valdez and Koob 2004). CRF, a 41 amino-acid residue peptide, is distributed throughout the brain, with high concentrations of cell bodies in the paraventricular nucleus of the hypothalamus and in areas of the extended amygdala (Bloom et al 1982). CRF is involved in mediating the physiological and behavioral responses to stress (Dunn and Berridge 1990; Vale et al 1981). Central administration of CRF mimics the behavioral responses to stress in rodents (Britton et al 1985; Dunn and File 1987; Sutton et al 1982; Swerdlow et al 1986), while administration of CRF antagonists reverses these effects (Britton et al 1986; Swerdlow et al 1989; Zorrilla et al 2002). CRF exerts its physiological and behavioral effects via both the hypothalamic-pituitary-adrenal (HPA) system, as well as an extrahypothalamic system which includes regions of the extended amygdala (Alheid and Heimer 1995; Dunn and Berridge 1990). The cellular effects of CRF are mediated by two types of high-affinity receptors, CRF_1 (Chang et al 1993; Chen et al 1993; Perrin et al 1993) and CRF_2 (Lovenberg et al 1995). Both receptors belong to the B_1 subgroup of G-protein coupled receptors and induce an increase in intracellular cyclic adenosine monophosphate (cAMP) upon ligand binding (Chen et al 1986; Giguere et al 1982). Genetic and pharmacological evidence indicates that the CRF_1 receptor is involved in mediating anxiety-like behavior in animals (Heinrichs et al 1997; Liebsch et al 1995; McElroy et al 2002; Smith et al 1998; Timpl et al 1998). However, the role of the CRF_2 receptor in mediating anxiety-related behaviors is less well understood. Indeed, some studies suggest that CRF_2 is more associated with appetite regulation and antistress-like effects (Pelleymounter et al 2000; Spina et al 1996; Valdez et al 2002a, 2003a).

The increased anxiety-like behaviors during ethanol withdrawal are believed to result, in part, from increased levels of extracellular CRF in extrahypothalamic brain regions (Merlo Pich et al 1995; Olive et al 2002), and central administration of CRF

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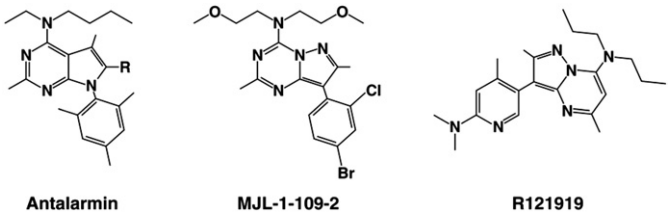

Figure 1. Chemical structures for antalarmin, MJL-1-109-2, and R121919.

antagonists can attenuate these behaviors (Baldwin et al 1991; Rassnick et al 1993; Valdez et al 2002b, 2003b). Ethanol-dependent animals also orally self-administer increased levels of ethanol during periods of withdrawal (O'Dell et al 2004; Roberts et al 2000; Valdez et al 2002b), effects which also likely result from enhanced CRF signaling in extrahypothalamic brain regions (Valdez et al 2002b). However, the specific receptor subtype of CRF involved in mediating these effects remains unknown. Because CRF_1 receptors play an important role in mediating anxiety-like behaviors (Heinrichs et al 1997; Liebsch et al 1995; McElroy et al 2002; Smith et al 1998; Timpl et al 1998; Zorrilla and Koob 2004), it was hypothesized that CRF_1 receptors also mediate the enhanced ethanol self-administration during withdrawal in dependent animals. Using an intermittent ethanol vapor exposure paradigm to induce ethanol dependence in male Wistar rats (O'Dell et al 2004), we show here that three separate, nonpeptide CRF_1 receptor antagonists, antalarmin, MJL-1-109-2, and R121919 (Figure 1), selectively reduce ethanol self-administration in ethanol-dependent animals during acute withdrawal. Importantly, none of these antagonists altered ethanol intake in nondependent rats. Because these drugs selectively reduce ethanol intake in dependent animals but not nondependent animals, CRF_1 antagonists may be a valuable new pharmacological treatment for alcoholism in humans.

Methods and Materials

Animals

Fifty-six adult male Wistar rats weighing 180 to 200 grams at the start of the experiment were obtained from Charles River Laboratory (Kingston, New York). Animals were housed two to three per cage with food and water available ad libitum. Lights were on a 12-hour light/dark cycle, lights on at 6:00 AM. All procedures met the guidelines of the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (National Research Council 1996).

Drugs

Ethanol (10% wt/vol) was prepared using 95% ethyl alcohol and water. The CRF_1 receptor antagonists antalarmin (*N*-butyl-*N*-ethyl-[2,5,6,-trimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-d] pyrimidin4-yl]-amine; K_i = 1.0; cLogP = 7.0), R121919 (3-[6-(dimethylamino)-4-methyl-pyrid-3-yl]-2,5-dimethyl-*N,N*-dipropyl-pyrazolo[2,3-a]pyrimidin-7-amine, also referred to as NBI-30775; K_i = 3.5; cLogP = 4.8), and MJL-1-109-2 (pyrazolo[1,5-a]-1,3,5-triazin-4-amine,8-[4-(bromo)-2-chlorophenyl]-*N,N*-bis(2-methoxy-ethyl)-2,7-dimethyl-(9Cl); K_i = 1.9, cLogP = 3), were synthesized by Drs. Kenner Rice and Mei-Jing Lee at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (Laboratory of Medicinal Chemistry) (Zorrilla and Koob 2004). Antalarmin was synthesized using modifications of the method of Chen (1994) and crystallized as described (Bornstein et al 1998). The CRF_1 receptor antagonists R121919 (Chen et al 2004) and MJL-1-109-2 (Jagoda et al 2003) were synthesized as described previously. The drugs were administered either subcutaneously (R121919 at 2 mL/kg) or intraperitoneally (IP) (antalarmin at 4 mL/kg and MJL-1-109-2 at 4 mL/kg). As antalarmin and MJL-1-109-2 are not as soluble as R121919, larger volumes were administered and delivered intraperitoneally as opposed to subcutaneously. These drugs cross the blood-brain barrier and block both the peripheral and central effects of CRF (Zorrilla and Koob 2004). Pharmacologically significant brain and plasma levels of antalarmin (Zorrilla et al 2002), R121919 (Chen et al 2004), and DMP696, an analog of MJL-1-109-2, (Yu-Wen et al 2003) have been reported. Receptor occupancy data for MJL-1-109-2 (Jagoda et al 2003) and R121919 (Heinrichs et al 2002) have also been reported previously. Vehicle for MJL-1-109-2 and R121919 was 20% wt/vol hydroxypropyl-β-cyclodextrin (HBC) (pH = 4.5) (Cargill Inc, Cedar Rapids, Iowa). Antalarmin was administered in .5% wt/vol carboxymethylcellulose (CBC) (pH = 4.5) (Sigma Chemical, St. Louis, Missouri). Drugs were systemically administered 1 hour (80 minutes for antalarmin) prior to self-administration testing.

Operant Ethanol Self-Administration

Ethanol self-administration was established in standard operant chambers (Coulbourn Instruments, Allentown, Pennsylvania) that were housed in sound-attenuated ventilated cubicles. Animals were trained to orally self-administer ethanol or water in a concurrent, two-lever, free-choice contingency. Syringe pumps (Razel Scientific Instruments, Stamford, Connecticut) dispensed ethanol or water into two stainless steel drinking cups mounted 4.0 cm above the grid floor in the middle of one side panel. Two retractable levers were located 4.5 cm to either side of the drinking cups. Fluid delivery and recording of operant self-administration were controlled by a microcomputer. Lever presses were not recorded during the .5 seconds in which the pumps were active. A continuous reinforcement (fixed ratio 1) schedule was used such that each response resulted in delivery of 0.1 mL of fluid.

Rats were trained to press a lever for ethanol using a modification of the sweetened solution fading procedure (Samson 1986). No fluid or food restriction period was employed. This training method culminates in rats consuming sufficient unsweetened 10% ethanol to produce pharmacologically relevant blood alcohol levels (Roberts et al 1999). Rats were initially trained to press a lever for a sweetened solution containing glucose (3% wt/vol) and saccharin (.125% wt/vol) (Sigma Chemical). Ethanol self-administration was initiated by adding ethanol (10%) to the sweetened solution for 4 to 5 days, followed by 4 to 5 days of 10% ethanol + .125% saccharin only. Finally, the animals received the 10% ethanol solution alone. During all training sessions, rats were also allowed to press for water on the opposite lever. The lever that produced water or ethanol was altered daily to prevent selecting rats biased toward one lever. The animals received daily (5 days per week) 30-minute access to ethanol for 20 to 25 days until stable rates of intake were observed. The criterion for stable baseline intake was ±20% across three consecutive sessions. Testing was performed at 8:00 AM (lights on at 6:00 AM).

Ethanol Vapor Chamber Procedure

To induce dependence, two standard rat cages were housed in separate, sealed, clear plastic chambers into which ethanol vapor was intermittently introduced. Ethanol vapor was created by dripping 95% ethanol (Central Stores, San Diego, California)