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Characteristics of ethanol-induced behavioral sensitization in rats: Molecular mediators and cross-sensitization between ethanol and cocaine

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ABSTRACT

Repeated exposure to drugs of abuse can induce a progressive increase in locomotor activity, known as behavioral sensitization. However, little is known about behavioral sensitization to ethanol. We examined whether ethanol could induce behavioral sensitization and investigated several molecular changes accompanying sensitization. We also assessed whether "cross-sensitization" occurred between ethanol and cocaine, another abused drug. Ethanol-induced sensitization was examined in rats after ethanol treatment (0.5 or 2 g/kg) for 15 days. The biochemical effects of low- or high-dose ethanol were examined in terms of *N*-methyl-D-aspartate (NMDA) receptor subunit phosphorylation or expression. Neuronal activity after ethanol treatment was assessed by measuring the level of early growth response (Egr-1) expression. Ethanol-induced behavioral sensitization was observed at the low dose (0.5 g/kg) but not the high dose (2 g/kg). Although acute treatment with the sensitizing dose of ethanol robustly increased Egr-1 protein and mRNA levels, the expression and phosphorylation of NMDA receptor subunits were not affected. The biochemical responses to ethanol seemed to be enhanced in ethanol-sensitized animals. Cross-sensitization between ethanol and cocaine was observed, which supports the hypothesis that there are commonalities among substances in the pathophysiology of substance dependence.

1. Introduction

Behavioral sensitization is a progressive enhancement in the locomotor-stimulating effect of a drug after repeated exposure (Robinson and Becker, 1986). It can serve as a model for drug dependence or psychosis (Robinson and Berridge, 1993; Wise and Bozarth, 1987). Drug-induced behavioral sensitization has been demonstrated for several abused drugs, especially psychostimulants, such as cocaine and amphetamines (Post and Rose, 1976; Shuster et al., 1977). These stimulants work by enhancing dopaminergic transmission, and the dopamine system plays a significant role in sensitization to stimulants (Martin-Iverson, 1991; Vanderschuren and Kalivas, 2000). Ethanol is also an abused drug that can induce severe dependence in its users. However, sensitization induced by ethanol is not well-studied in rats (Goldstein et al., 1992; Hoshaw and Lewis, 2001), which may be due to the fact that ethanol does not have strong stimulating effects compared to psychostimulants. Ethanol has a biphasic effect on locomotor activity (Masur et al., 1986; Phillips et al., 1995; Phillips et al., 1997). Locomotor activity is decreased by high doses of ethanol (Duncan and Baez, 1981; Pohorecky, 1978), whereas it is increased by lower doses (Gingras and Cools, 1996; Moore et al., 1993). This dose-dependent change in effect could affect the development of sensitization. However, it has not been well established that ethanol induces sensitization.

The first aim of the present study was to test whether repeated treatment with ethanol could induce behavioral sensitization, and, if so, whether its development was dependent on the dose. Previous studies have suggested that *N*-methyl-D-aspartate (NMDA) receptors are necessary for the induction of sensitization to stimulants (Landa et al., 2014; Sripada et al., 2001). Although behavioral sensitization to ethanol is not dependent upon the sensitivity of NMDA receptors (Meyer and Phillips, 2007), the stimulatory effects of ethanol may be related to NMDA receptor activity (Phillips and Shent, 1996). Thus, the present study examined dose-dependent changes in NMDA receptor subunit protein expression and phosphorylation following the acute administration of ethanol. To assess neural activity independent of the behavioral response, early growth response (Egr-1) protein and mRNA

Abbreviations: ANOVA, analysis of variance; CNS, central nervous system; Egr-1, early growth response-1; GABA, gamma-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; OD, optical density

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levels (Knapska and Kaczmarek, 2004) were examined. Because drug-related neuronal activity is an important aspect of the neurobiological substrates underlying addiction (Torres and Horowitz, 1996; Vilpoux et al., 2009), these molecules were examined in both ethanol-naïve and -sensitized animals.

Although ethanol works primarily as an antagonist of NMDA receptors and an agonist of GABA receptors (Koob et al., 1998a), it has been suggested that ethanol could activate the dopaminergic system via opioid mediation (Ortiz et al., 1995). This indicates that there are similarities in the actions of ethanol and cocaine in terms of the dopaminergic, glutamatergic, and GABAergic systems (Koob et al., 1998b; Marinho et al., 2015; Morice et al., 2010; Wise, 1998). Thus, a second aim of this study was to investigate whether behavioral sensitization induced by one drug could result in the sensitization of a subject to another drug: that is, whether cross-sensitization between the CNS depressant ethanol and the CNS stimulant cocaine would develop. The cross-sensitization of ethanol and cocaine could provide evidence that some overlap exists in the mechanisms of sensitization induced by both drugs (Itzhak and Martin, 1999; Lessov and Phillips, 2003).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (weight: 150–200 g; KoaTech, Gyeonggi-do, Korea) were housed under a 12/12-h light/dark cycle with food and water available ad libitum. The present study was approved by the Institutional Animal Care and Use Committee at Seoul National University Hospital (SNUH-IACUC) and the animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International (#001169) in accordance with the Guide for the Care and Use of Laboratory Animals 8th edition, National Research Council (NRC, 2010).

2.2. Drugs

Ethanol (Merck, Whitehouse Station, NJ, USA, cat. #1.00983.1011) was diluted to 20% (w/v) in 0.9% saline and administered in doses of 0.5 or 2 g/kg. Cocaine hydrochloride (15 mg/kg; Belgopia, Louvain-La-Neuve, Belgium) was dissolved in 0.9% saline. All drugs were administered intraperitoneally (IP). To minimize contextual effects on the development of behavioral sensitization and to focus only on drug-dependent sensitization, with the exception of the days of behavioral measurement, all drugs were given in the animal's home cage. On behavioral testing days, the animals were transferred to the test cage, and drugs were administered after a habituation period (30 min) in the cage.

2.3. Schedule of drug administration

Five sets of experiments were conducted. The first set was performed to examine the acute effects of ethanol. We examined the expression and phosphorylation of NMDA receptor subunits and the expression of the Egr-1 gene in response to varying doses of ethanol. The animals were randomly assigned to three treatment groups (n = 5 or 6 per group): 0 (control, saline only), 0.5 g/kg (low-dose), and 2 g/kg (high-dose) groups. They were decapitated at 30 min after the treatment and the brains were dissected.

The second set was performed to investigate whether ethanol induced behavioral sensitization. The animals were randomly assigned to three groups (n = 5 or 6 per group). Ethanol or saline was administrated for 3 weeks (injected 15 times, 5 times per week from Monday to Friday from 10:00 AM until 4:00 PM). Following the first, fifth, tenth, and fifteenth injections, the locomotor activity of the rats was assessed for 60 min to assess sensitized behavior. Sensitization was defined as a statistically significant increase in locomotor activity compared with that of the control group. In this experiment, we sought to determine the dose and number of treatments required to induce sensitization.

Because the second experiment indicated that ethanol could induce behavioral sensitization, the third set of experiments was performed to measure the expression and phosphorylation of NMDA receptor subunits and expression of the Egr-1 gene in response to ethanol in the sensitized state. The sensitized (treated with 0.5 g/kg ethanol 15 times) and saline (treated with saline 15 times) groups were further divided into two subgroups that received either 0.5 g/kg of ethanol (E-E and S-E) or saline (E-S and S-S) the next day. Locomotor activity was not measured after the final challenge, and the rats were decapitated 30 min thereafter.

The fourth and fifth experiments were designed to investigate whether ethanol and cocaine cross-sensitize. In the fourth set of experiments, two groups of rats received cocaine or saline for 5 consecutive days. The treatment regimen for sensitization to cocaine was selected based on a previous study from our research group (Xu and Kang, 2016). After the fifth treatment, the rats were further divided into two subgroups, one of which was treated with a sensitizing dose of ethanol (0.5 g/kg) or saline (n = 6/subgroup). Following the drug challenges, the locomotor activity was recorded for 30 min to test for sensitized behavior. In the fifth set of experiments, two groups of rats received ethanol or saline for 3 weeks (injected 15 times). After the 15th injection, the rats were further divided into two subgroups and treated with cocaine (15 mg/kg) or saline and their locomotor activities were then measured (n = 6/subgroup).

2.4. Measurement of locomotor activity

Locomotor activity was measured using a video-tracking apparatus (Activity Monitor Ver. 5.0; Med-Associates, St. Albans, VT, USA) in a transparent acrylic box (42 × 42 × 30 cm) located in a sound-attenuated test room. Animals were placed in the box and allowed to freely explore the box during a 30-min habituation period. After the habituation period, animals were challenged with drugs, and the locomotor activity was recorded in 5-min bins for another 30- or 60-min period.

2.5. Immunoblot analysis

Brains were dissected on ice plates; the prefrontal cortex and dorsal striatum were homogenized in lysis buffer containing 50 mM Tris buffer (pH 7.4), 150 mM NaCl, 1 mM DTT, 4 mM EGTA, 10 mM EDTA, 100 mM β-glycerophosphate, 40 mM NaF, 4 mM sodium vanadate, 15 mM sodium pyrophosphate, 1 mM PMSF (Sigma-Aldrich, St. Louis, MO, USA), 0.2% NP40, and a protease inhibitor cocktail (Roche, Mannheim, Germany). This procedure has been described in detail previously (Park et al., 2010). After centrifugation (20,000 rpm, 20 min), protein in the supernatants were quantified using the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA) and boiled in Laemmli sample buffer. Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Whatman, Dassel, Germany). The membrane was blocked with blocking buffer containing 5% skim milk in TBS-T (0.1% Tween 20 in TBS) for 1 h at room temperature and then incubated with primary antibodies overnight at 4 °C. The membranes were then incubated with secondary antibodies for 1 h at room temperature. Primary antibodies against NR1, phospho-S896-NR1, NR2A, NR2B, Egr-1 (Cell Signaling Technology, Danvers, MA, USA), or β-actin (Sigma-Aldrich) were used at dilutions of 1:1000 to 1:30,000. Anti-rabbit or mouse IgG was used as the secondary antibody (Jackson Immuno Research Laboratories Inc., West Grove, PA, USA). Signals were detected using an enhanced chemiluminescence system (Pierce Biotechnology, Rockford, IL, USA). Signals on developed X-ray films were quantified with the TINA 2.10G software package (Raytest, Straubenhardt, Germany).