Neuroscience 169 (2010) 125–131

N-METHYL-D-ASPARTIC ACID RECEPTORS ARE ALTERED BY STRESS AND ALCOHOL IN WISTAR–KYOTO RAT BRAIN

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Abstract—Previous studies have shown that the Wistar–Kyoto (WKY) rat strain is more sensitive to stressors and consumes significant quantities of alcohol under basal as well as stressful conditions when compared to other strains. Given that the glutamate neurotransmitter system has been implicated in depression and addiction, the goals of the present study were to investigate the effects of stress and stress-alcohol interactions on N-methyl-D-aspartate (NMDA) receptors in the rat brain. Thus this study measured the binding of [^{3}H] MK-801 to NMDA receptors in the prefrontal cortex (PFC), caudate putamen (CPu), nucleus accumbens (NAc), hippocampus (HIP) and basolateral amygdala (BLA) in WKY rats in comparison to the Wistar (WIS) rat strain. Our results suggested that while voluntary alcohol consumption did not alter NMDA receptors in the PFC, CPu or NAc in either rat strain, it increased NMDA receptors in the HIP and BLA in both strains. In contrast, chronic stress increased NMDA receptors in the PFC, CPu, NAc in WKY rats but not in WIS rats. Chronic stress also decreased NMDA receptors in the HIP and increased NMDA receptors in the BLA in both strains. Alcohol co-treatment with stress increased NMDA receptors in the PFC, CPu and NAc in WKY rats but not in WIS rats. Interestingly, while alcohol co-treatment did not reverse stress induced decreases in NMDA receptors in the HIP, it reduced the binding of NMDA receptors in the BLA to control levels in both strains. Thus it appears that NMDA receptors in the PFC, CPu and NAc may be more sensitive to the effects of stress and could be implicated in the stress-induced alcohol consumption behavior seen in WKY rats. In contrast, NMDA receptors in the HIP and BLA may reflect an adaptive response and may not be responsible for the stress susceptible phenotype of the WKY rat strain.

Key words: depression, animal model, [^{3}H] MK-801 binding, glutamate.

Numerous studies have suggested that negative emotional state (e.g. depression) and exogenous stressful stimulation contribute to the development of alcoholism in human subjects (Brown et al., 1995) and experimental animals (Rockman et al., 1987; Coffey et al., 2002). According to the National Comorbidity Surveys (NSDUH2005) and other related investigations, alcohol dependency most commonly co-occurs with depressive disorder (Kessler et al., 1997; Jane-Llopis and Matytsina, 2006). Treatment with antidepressant drugs has been shown to reduce depressive symptoms as well as decrease alcohol consumption in alcoholic patients (Brown et al., 1997; Cornelius et al., 1997). Although the exact mechanism responsible for the possible linkage between stress and psychological disorders is not well understood, it has been suggested that the interaction between genetic-predisposition and environmental stressors influences the presentation of conditions such as major depression as well as addiction (Zubin and Spring, 1977; Kendler et al., 1995; Parnas, 1999).

It is well known that glutamate homeostasis and neurotransmission are disrupted in major depressive disorder (Paul and Skolnick, 2003; Feyissa et al., 2009). Stress-induced modulation of glutamate has been proposed to contribute to the etiology and progression of depressive illness (Moghaddam, 2002). Stress exposure increases excitatory amino acid neurotransmission in the prefrontal cortex (PFC), caudate–putamen (CPu), hippocampus (HIP) and amygdala (Lowy et al., 1993; Moghaddam, 1993; Bagley and Moghaddam, 1997); brain regions that are implicated in stress, depression and reward. Alcohol consumption affects brain functions by interacting with several neurotransmitter systems including the glutamatergic system of which the N-methyl-D-aspartic acid (NMDA) receptor is a major molecular target (Alele and Devaud, 2005; Heinz et al., 2005; Larsson et al., 2005; Raeder et al., 2008; Ridge et al., 2008). A glutamatergic hypothesis of human alcoholism suggests that neuropsychological and pathological effects of alcohol may be mediated through the glutamatergic system (Samson and Harris, 1992; Tsai et al., 1995), especially the NMDA type of glutamate receptors (Nagy et al., 2005).

The Wistar Kyoto (WKY) rat strain has been proposed as an animal model of depressive behavior (Lopez-Rubalcava and Lucki, 2000; Paré, 1989a,b; Redei et al., 1994; Tejani-Butt et al., 2003). Several studies have noted that WKY rats differ from other strains in their behavioral, physiological, and neuroendocrine responsiveness to environmental as well as pharmacological challenges (Lopez-Rubalcava and Lucki, 2000; Redei et al., 1994; Tejani-Butt et al., 1994, 2003). Exposing WKY rats to stress stimulation results in behaviors which resemble human depressive behavior, such as anhedonia (Paré, 1994a), psychomotor retardation (Paré, 1994b), ambivalence (Paré, 1993), and negative memory bias (Paré, 1996). We have previously suggested that the WKY rat strain may represent a suitable model for studying the neurochemical mechanisms underlying depressive behavior and in-

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Abbreviations: AL, alcohol treatment; BLA, basolateral amygdala; CPu, caudate–putamen; CS, chronic-stress; DA, dopamine; HIP, hippocampus; NAc, nucleus accumbens; NMDA, N-methyl-D-aspartic acid; PFC, prefrontal cortex; WIS, Wistar; WKY, Wistar Kyoto.

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doi:10.1016/j.neuroscience.2010.05.003

creased alcohol consumption (Paré et al., 1999; Jiao et al., 2006). Given the involvement of NMDA receptors in the effects of alcohol as well as stress, the present study investigated the effects of alcohol consumption, stress and stress-alcohol interactions on the binding of [^{3}H] MK-801 to NMDA receptor sites in the brains of WKY rats and compared them to a control Wistar (WIS) rat strain.

EXPERIMENTAL PROCEDURES

Animals

WIS rats were purchased from Harlan (Indianapolis, IN, USA). WKY rats were obtained from Charles River Laboratories (Kingston, NY, USA). Age matched male WKY and WIS rats (6–8 months) were used in this study. Animals were handled according with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and with the Perry Point VAH Institutional Review Committee approval. All efforts were made to minimize the number of animals used and their suffering. Animals were individually housed at 22 °C and placed on a 12-h light/dark cycle. Both strains were assigned into four groups (n=8): (1) control group (Control), (2) no stress with 24-day alcohol treatment (AL), (3) 24-day chronic-stress (CS) and (4) 24-day chronic-stress with 24-day alcohol treatment (CS-AL).

Alcohol treatment

The alcohol procedure established by Sandbak and Murison (1996) was used with minor modification. Animals from alcohol groups were given access alcohol or water by offering two sipper-type drinking tubes with tap water in one and alcohol solution in the other. The position of the tubes was switched daily (left/right) to eliminate the possibility of location preference. On the other hand, control group was offered two sipper tubes containing water. A 3% solution was presented during the first (1–7 days), a 5% solution on the second 7-day (8–14 days) period, and a 7% solution was presented during the rest of time (15–24 days). Body weight and alcohol consumption was recorded daily at the same time.

Chronic mild stress procedure

A chronic mild stress procedure with a repeated-novel-stressor regimen, based on the procedure established by Katz et al. (1981), with minor modifications (Tejani-Butt et al., 1994), was used in this study. Experimental animals were exposed to a schedule of different daily stressors. The elements of the schedule consists of a scrambled foot shock (1.0 s, 1.0 mA scrambled foot shock presented with a variable interval of 10 s between shocks in a 20×20×25 cm^3 stainless steel cage for 30 min), food deprivation (remove food from home cage for 40 h), cold swim (forced swim in 4 °C water for 5 min), water deprivation (remove water bottle 24 h), restraint (placing the rat in ¼-in hardware cloth tube with a diameter of 38 cm and 18 cm long for 2 h), shaker stress (animal was in home cage and placed on shaker platform for 15 min), restraint (rat was placed in a 5 mm hardware cloth tubes with a diameter of 3.8 and 18 cm long for 2 h), heat stress (exposed to 40 °C ambient temperature in a drying oven for 5 min), reverse light/dark cycle (artificial light on between 18.00 and 06.00 h); switch cage mate; increased housing density (rats housed five per 18×18×32 cm^3 cage for 24 h). The order of stress administration is described in Table 1. Body weight was recorded daily at the same time.

Table 1. Chronic stress schedule

Days	Stress treatment
1	Foot shock
2	Food deprivation
3	Cold swim
4	Water deprivation
5	Restraint
6	No stress
7	Shaker stress
8	Heat stress
9	Food deprivation
10	Heat stress
11	Restraint
12	Reverse L/D cycle
13	Cold swim
14	Foot shock
15	Switch cage mate
16	Food deprivation
17	Reverse L/D cycle
18	Increase housing density
19	Foot shock
20	Water deprivation
21	Heat stress
22	Food deprivation
23	Shaker stress
24	Switch cage mate

Stress-alcohol treatment

The stress-alcohol group received the same stress procedure as described above, but received free access to water or alcohol (as described above). Body weight was recorded daily at the same time (Fig. 1). Alcohol consumption was recorded daily at the same time and has been reported previously (Yaroslavsky and Tejani-Butt, 2010).

Brain section preparation

On day 25, all animals were sacrificed by rapid decapitation and the brains were removed immediately, dipped into −20 °C isopentane (Sigma, St. Louis, MO, USA) and stored at −80 °C until use. All of the brain tissue sections (16 μm) were cut at −18 °C in a cryostat microtome (The Vibratome Company, St. Louis, MO, USA) according to the Brain Atlas of Paxinos and Watson (1998) and mounted onto gelatin-coated microscope slides. Sections from plate 12 and 30 including PFC, CPu and NAc, HIP and basolateral amygdala (BLA) were examined in the current study.

[^{3}H] MK-801 binding assay

NMDA receptor autoradiography of [^{3}H] MK-801 (Sigma Aldrich; 27.5 Ci/mmol) was performed as previously described (Lei et al., 2009). Briefly, brain sections were thawed to room temperature and pre-washed in 50 mM Tris–HCl buffer (pH 7.4) for 30 min at 4 °C. Then, the sections were incubated in 50 mM Tris–HCl buffer containing 15 nM [^{3}H] MK-801 and 30 μM glutamate/15 μM glycine for 120 min at room temperature, rinsed for 30 min with cold 50 mM Tris–HCl buffer, dipped once in ice cold distilled water, and immediately dried in a stream of cool air. Non-specific binding was measured in the presence of 50 μM non-radioactive MK-801 and was less than 10% of total binding. Dried tissue sections were exposed to autoradiography film. Following a 4-week exposure period at 4 °C, the film was developed in Kodak D19, at room temperature.