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Role of cortical alpha-2 adrenoceptors in alcohol withdrawal-induced depression and tricyclic antidepressants

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ABSTRACT

Introduction: Although a role for alpha-2 adrenoceptors (alpha-2 ARs) in alcohol use disorder (AUD) and depression is suggested, very little information on a direct interaction between alcohol and these receptors is available.

Methods: In this study adult female Wistar and Wistar-Kyoto (WKY) rats, a putative animal model of depression, were exposed to alcohol vapor 3 h daily for 10 days (blood alcohol concentration ~150 mg%) followed by daily injection of 10 mg/kg of imipramine (IMP, a selective norepinephrine NE/serotonin reuptake inhibitor) or nomifensine (NOMI, a selective NE/dopamine reuptake inhibitor). On day 11 animals were tested for open field locomotor activity (OFLA) and forced swim test (FST) and were sacrificed 2 h later for measurement of alpha-2 ARs densities in the frontal cortex and hippocampus using [3H]RX 821002 as the specific ligand.

Results: Chronic alcohol treatment increased the immobility in the FST, without affecting OFLA in both Wistar and WKY rats, suggesting induction of depressive-like behavior in Wistar rats and an exacerbation of this behavior in WKY rats. Alcohol treatment also resulted in an increase in cortical but not hippocampal alpha-2 ARs densities in both Wistar and WKY rats. The behavioral effects of alcohol were completely blocked by IMP and NOMI and the neurochemical effects (increases in alpha-2 ARs) were significantly attenuated by both drugs in both strains.

Conclusions: The results suggest a role for cortical alpha-2 ARs in alcohol withdrawal-induced depression and that selective subtype antagonists of these receptors may be of adjunct therapeutic potential in AUD-depression co-morbidity.

1. Introduction

A significant co-morbid expression of alcohol use disorders (AUD) and depression is evident in epidemiological studies (Boschloo et al., 2011; Dixit and Crum, 2000; Iovieno et al., 2011; Lai et al., 2015; Rodgers et al., 2000; Schuckit, 2006; Spak et al., 2000). Among the AUD treatment population, co-morbid depression can affect as much as 50% of people (Swendsen and Merikangas 2000). Similarly, depression treatment populations may have up to 40% life-time probability of developing AUD (Grant et al., 2004; Jane-Llopis and Matytsina, 2006). Co-occurrence of AUD and depression results in greater disease burden than each disorder alone (Gadermann et al., 2012). Such dual diagnosis is an important clinical assessment since treatment outcome for either condition, if considered separately, may not be fully adequate (Iovieno et al., 2011). Interestingly, pharmacological treatment of the depressive symptoms results in a better treatment outcome for AUD (Kessler et al., 1997; Schuckit et al., 1997). Likewise, treatment of primary AUD results in rapid reduction in depressive symptoms (Brown and Schuckit, 1988). Indeed, 80% of AUD patients with major depression no longer present depressive symptoms after 2 weeks of sobriety (Dackis et al., 1986). However, if unmanaged, depressive symptom especially during alcohol withdrawal can lead to relapse and increased alcohol intake (Dixit and Crum, 2000; Hodgins et al., 1995; Johanson and Fischman, 1989; Schulteis et al., 1995).

A positive relationship between depressive symptoms and voluntary alcohol intake has also been observed in animal models. Thus, Wistar-Kyoto (WKY) rats, considered a putative and non-induced animal model of depression, voluntarily consume more alcohol than their control counterparts, Wistar rats or Sprague-Dawley rats (Jiao et al., 2006; Paré et al., 1999; Yaroslavsky and Tejani-Butt, 2010). Conversely, alcohol

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preferring (AA) rats may exhibit depressive-like characteristics following voluntary alcohol intake compared to alcohol non-preferring (ANA) rats (Viglinskaya et al., 1995). Although various theories have attempted to explain the association between AUD and depression, it appears that a number of factors, including genetic predisposition and alterations in neurochemical substrates, such as the noradrenergic system, may contribute to this co-morbidity (Balsamo et al., 2016; Bravo et al., 2017; Donadon and Osório, 2016; Getachew et al., 2010; Jung et al., 2016; Kalejaiye et al., 2013; Merikangas and Gelernter, 1990; Overstreet et al., 2005; Rezvani et al., 2002, 2007; Rincon-Hoyos et al., 2016).

Alpha adrenergic receptors (alpha ARs) are one of the major classes of G protein-coupled receptors for norepinephrine (NE) that are predominantly located pre-synaptically, but are also present post-synaptically in the central nervous system (Bylund, 1988, 1995; Giovannitti et al., 2015; U'Prichard et al., 1979). There are two subtypes of alpha ARs (alpha 1 and alpha 2) (Bylund, 1995). In humans, three subtypes of alpha-2 ARs (alpha 2A, alpha 2 B and alpha 2C) and in rats, four subtypes (alpha 2A, alpha 2B, alpha 2C and alpha 2D) have been identified. In the rat, alpha 2D is a species variation of human alpha 2A. These receptors play an important role in regulating the neuronal release of NE through presynaptic feedback inhibition and may be at least partially responsible for pathogenesis and symptomatic expression of depressive illness. Thus, a variety of antidepressants (e.g., desipramine) and other treatments of depression (e.g., electroconvulsive shock therapy) are associated with decreases in the density and sensitivity of central alpha-2 ARs (Barturen and Garcia-Sevilla,1992; Cohen et al., 1980; Invernizzi and Garattini, 2004; Pilc and Vetulani (1992); Smith et al., 1992; Tanaka and Telegdy, 2014). Interestingly, manipulations of the alpha-2 ARs may also affect alcohol intake. Hence, yohimbine, an alpha-2 AR antagonist can reinstate alcohol seeking after extinction (Funk et al., 2016; Marinelli et al., 2007). On the other hand, alpha-2_A AR agonists that may decrease availability of NE reduce alcohol consumption (Fredriksson et al., 2015; Opitz, 1990; Rasmussen et al., 2014). However, very few studies have investigated direct effects of alcohol on apha-2 ARs, particularly in relation to depressive-like characteristics.

A major goal of this study was to investigate the effects of alcohol as well as two tricyclic antidepressants (imipramine, a selective norepinephrine/serotonin NE/5HT uptake inhibitor, and nomifensine, a selective NE/dopamine DA uptake inhibitor) on alpha-2 ARs in Wistar (control) and WKY rats. We hypothesized that alcohol withdrawal-induced depressive-like behavior will be associated with an increase in alpha-2 AR densities in two important brain regions, namely the frontal cortex and the hippocampus. Moreover, we anticipated that chronic treatment with antidepressants that affect the noradrenergic system would normalize the behavioral as well as the neurochemical effects of alcohol on alpha-2 ARs.

2. Materials and method

2.1. Animals

Adult female WKY rats, approximately 4 months old (Harlan, IN, USA) were used throughout the study. We chose female rats because: 1.) there exists a higher incidence of depression in women, but there are sparse inclusion of female animals in such studies (Kessler et al., 1993; Nolen-Hoeksema, 1990; Weissman et al., 1996); 2.) as in humans, there is also a higher incidence of depressive-like behavior in females compared to male WKY rats (Paré and Redei, 1993); 3.) both female rats (Carrier and Kabbaj, 2013; Kokras et al., 2015) and mice (Franceschelli et al., 2015) show enhanced response and greater sensitivity to antidepressant treatments compared to their male counterparts. Although menstrual cycle may have an important role in human behavioral outcome, it should be noted that the estrous cycle in rats is relatively very short (4 days, Spornitz et al., 1999), and that rats tend to synchronize their estrous cycle when housed in group or close proximity (Alekhina et al., 2015; McClintock, 1978) . In addition, use of appropriate controls, as used in this study, can reduce the chance of false positive or negative results when using female rats.

Animals were housed in groups of three or four in standard polypropylene shoebox cages (42 × 20.5 × 20 cm) on hardwood chip bedding (alpha-dry) in a room designated for female rats. Throughout the experiment, animals had access to food (Harlan Tek Lab) and water ad libitum. The room was maintained at 24–26 °C at 51–66% relative humidity, on a 12-h reversed light/dark cycle (lights on at 1900 h). All experiments were carried out in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee.

In order to acclimate the subjects to the housing conditions, animals arrived at least one week prior to initiation of any experiment. During this period, they were gentled once daily in order to minimize any stress effects that might result from routine handling. All behavioral tests were carried out in the early portion of dark phase between 09:00 AM and 12:00 PM using a red light as source of illumination. The dark phase was chosen to coincide with the animals' nocturnal nature (i.e., being active during the dark phase). Each experimental group consisted of 7–8 animals.

2.2. Vapor ethanol exposure and drug treatment

Inhalation chambers were used to expose the animals to ethanol (La Jolla Alcohol Research Inc., La Jolla, CA, USA). Briefly, 95% ethanol (EtOH) was pumped at regulated rate from 5-gallon reservoir via a peristaltic pump to a 5000 ml Erlenmeyer vacuum flask that was kept on a warming tray (52 °C). EtOH was then volatilized and mixed with pressurized air. The flow of this mixture was controlled by a pressure gauge as it was delivered to individual chambers. The parameters used in EtOH vapor exposure were: air pressure ≈5 psi, airflow rate ≈ 15–20 l/min and EtOH flow rate = 60 ml/hr. A control group received only air via a system that exactly mirrored the experimental condition. The animals were exposed to EtOH vapor for 3 h daily, during which, a blood alcohol concentration (BAC) of approximately 150 mg% was maintained. The inhalation chamber has the advantage of easily achieving and maintaining the targeted BAC (Kliethermes et al., 2004). Moreover, the variability in the alcohol concentration between similarly controlled chambers is minimal (Lee et al., 2000; Getachew et al., 2008, 2010).

USP 200 proof EtOH was purchased from VWR Scientific Products (Bridgeport, New Jersey, USA) and was diluted down (95% v/v) with distilled water. Imipramine (IMP) HCl and nomefensine (NOMI) maleate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), were dissolved in saline and injected intraperitoneally (i.p.) (10 mg/kg) in a volume of 1 ml/kg, immediately after removal from the vapor chamber each day. Control animals were injected with the same volume of physiological saline. To verify the "depressogenic" effect of alcohol, a separate control group was exposed to air in an exactly same set up followed by saline injection only. The dosing of drugs was chosen based on works of others as well as ours that have been reported as optimally effective antidepressant doses (Tejani-Butt et al., 2003; Getachew et al., 2008).

2.3. Behavioral evaluations

2.3.1. Open field locomotor activity (OFLA) monitoring

On day 11, approximately 16 h after the last alcohol exposure, animals were tested in an open-field activity-monitoring cage (27 × 27 × 20.3 cm, Med Associates, Inc., St. Albans, VT) for 10 min, where ambulatory counts representing the number of infrared beam interruptions were recorded (Getachew et al., 2010).

2.3.2. Forced swim test (FST)

Immediately following the open field activity test, each animal was