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Chronic ethanol consumption increases dopamine uptake in the nucleus accumbens of high alcohol drinking rats

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Abstract

Past research has indicated that chronic ethanol exposure enhances dopamine (DA) neurotransmission in several brain regions. The present study examined the effects of chronic ethanol drinking on dopamine transporter (DAT) function in the nucleus accumbens (Acb) of High-Alcohol-Drinking replicate line 1 (HAD-1) rats. HAD rats were given concurrent 24-hr access to 15% ethanol and water or water alone for 8 weeks. Subsequently, DA uptake and the $V_{\max}$ of the DAT were compared between the two groups using homogenates of the nucleus accumbens. DA uptake was measured following a 2 minute incubation at 37°C in the presence of 8 nM [³H]DA. For kinetic analyses, DA uptake was assessed in the presence of 5 concentrations of [³H]DA ranging from 8nM to 500nM. Analyses of the data revealed a significant increase in DA uptake in the ethanol group compared to water controls. Kinetic analyses revealed the change in DA uptake to be a consequence of an increase in the $V_{\max}$ of transport. These findings demonstrate that chronic free-choice oral ethanol consumption in HAD-1 female rats increases DA uptake in the Acb by increasing the $V_{\max}$ of the transporter. However, it is not known whether the ethanol-induced change in $V_{\max}$ is caused by differences in the actual number of available transporter sites or from a difference in the velocity of operation of a similar number of transporters. Overall, the data indicate that chronic ethanol consumption by HAD-1 rats produces prolonged neuroadaptations within the mesolimbic DA system, which may be important for the understanding of the neurobiological basis of alcoholism.

1. Introduction

Selective breeding for high alcohol preference and intake in rats has produced a number of animal models of alcoholism. Although less characterized than the alcohol-preferring (P) rats (c.f. Murphy et al., 2002; McBride & Li, 1998), high-alcohol drinking (HAD-1 and HAD-2) replicate lines are engineered from heterogeneous N/Nih stock rats and are selectively bred on the basis of their preference for a 10% (v/v) ethanol solution with water and food concurrently available (Li et al., 1993). HAD rats voluntarily consume similar amounts of ethanol as the P rats during adolescence (McKinzie et al., 1998) and adulthood (Li et al., 1993). In addition, HAD rats will orally self-administer ethanol solutions in an operant paradigm (Ritz et al., 1994; Samson et al., 1998; Oster et al., submitted). These models of alcoholism have been evaluated on how well they reflect the various behavioral phenotypes associated with

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alcoholism. In human studies of alcoholism, a low sensitivity to ethanol's effects appears to be predictive of future alcoholism (Schuckit, 1994). Overall, rodent studies that have examined ethanol sensitivity in the selected lines have shown that the alcohol-preferring rat lines are typically less sensitive to ethanol's effects than the –nonpreferring rat lines (Froehlich & Wand, 1997; Kurtz et al., 1996; Lumeng et al., 1982; Stewart et al., 1992).

The mesolimbic dopamine system has been hypothesized to mediate the reinforcing actions of drugs of abuse and ethanol. Acute administration of ethanol directly alters the mesolimbic DA system. Alcohol-preferring animals exhibited a greater dopaminergic response to acute ethanol administration than –nonpreferring animals (Katner & Weiss, 2001). Electrophysiological studies demonstrated that ethanol administration increased the firing rate of ventral tegmental area (VTA) DA neurons *in vivo* (Gessa et al., 1985) and *in vitro* (Brodie et al., 1990; 1999). Systemic administration of ethanol has been shown to increase extracellular DA levels in the Acb and VTA (Di Chiara & Imperato, 1985; Imperato & Di Chiara, 1986; Yim & Gonzales, 2000; Yoshimoto et al., 1992a, b)). Additionally, microdialysis studies demonstrated an increase in DA release in the Acb following oral self-administration of ethanol (Katner et al., 1996; Melendez et al., 2002; Weiss et al., 1993). Finally, preliminary data indicate that microinjections of ethanol into the posterior VTA increased extracellular concentrations of DA in the Acb (Toalston et al., 2005). Chronic ethanol consumption can modulate the effects of ethanol within the mesolimbic DA system. Brodie (2002) demonstrated that chronic ethanol exposure in C57BL mice increased the sensitivity of the VTA DA neurons to the stimulating effects of ethanol. Repeated twice daily injections (21 days) of ethanol (3.5 g/kg) increased the stimulating effects of ethanol on VTA DA neurons while reducing the inhibitory influence of GABA (Brodie, 2002). Adolescent ethanol consumption augmented the increase in extracellular DA levels in the Acb following peripheral administration of ethanol during adulthood (Sahr et al., 2004). Additionally, as indicated by the no-net flux microdialysis technique, adolescent ethanol consumption may result in an increase of DA uptake in the Acb during adulthood (Sahr et al., 2004). Chronic, ongoing ethanol consumption increased the sensitivity of the posterior VTA to the reinforcing effects of EtOH (Rodd et al., 2005a). Furthermore, P rats exposed to repeated cycles of alcohol access and abstinence (the repeated “alcohol deprivation effect” model) and deprived of ethanol for two months prior to testing, self-administered ethanol directly into the posterior VTA at lower concentrations than chronically drinking and naïve P rats (Rodd et al., 2005b). These studies indicate that ethanol exposure alters the mesolimbic DA system response to ethanol, and that chronic ethanol consumption produces long-term neuroadaptations in the mesolimbic DA system. It should be noted, however, that with the exception of the Brodie (2004) study, the aforementioned studies examined the effects of chronic ethanol exposure on DA systems in the alcohol-preferring rats. Although many studies have examined the effects of chronic ethanol exposure on rodents, others have used monkey models of alcoholism. Budygin et al. (2003) examined the effects of chronic ethanol exposure on the striatal DA neurons of macaque monkeys using fast-scan cyclic voltammetry. They reported that DA uptake rates were significantly higher in the caudate of ethanol-drinking monkeys than naïve monkeys. Uptake rates were estimated by measuring the rate of DA disappearance following electrical stimulation.

Taken together, these studies indicate that acute ethanol exposure acts within the mesolimbic DA system, on-going ethanol consumption alters the effects of ethanol within the mesolimbic DA system, and that past experience with ethanol consumption produces prolonged neuroadaptations within the mesolimbic DA system. The current study examined the effects of chronic ethanol consumption on DA uptake in HAD rats. Given the results of Sahr et al. (2004), it was hypothesized that chronic ethanol exposure would lead to an increase in DA uptake in the Acb of HAD-1 rats.

2. Materials and Methods

The experimental procedures used in this study were in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute on Drug Abuse, National Institute of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, 1996). All procedures were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine.

2.1. Animals

Subjects were ethanol-naïve female HAD-1 rats that weighed between 94–128 g at the start of the experiment (age range 80–110 days of age). Rats were supplied by Indiana University School of Medicine breeding colonies. Previous studies have shown no effect of the estrous cycle on ethanol intake or on the expression of the alcohol deprivation effect (McKinzie et al., 1998).

Animals were double-housed throughout the experiment in plastic tubs in a temperature (21°C) and humidity (50%) controlled vivarium maintained on a reverse 12 hr. light/dark cycle (lights off at 0700 hr). Food and water were available *ad libitum*. Alcohol intake was determined every other day, and the volume consumed was divided by four in order to obtain an estimate of the amount of ethanol consumed per rat, per day. The uptake data obtained from alcohol-drinking rats that were housed together did not exhibit significant variance, and therefore the data were not pooled, but were analyzed separately.

2.2. Experimental procedure

Following one week of acclimation to the vivarium, rats were divided into an ethanol group and a water control group (n=4 per group). The ethanol group was allowed unlimited 24 h/day two-bottle, free choice access to 15% v/v ethanol and water for 8 consecutive weeks. To assess the acquisition of ethanol drinking, ethanol and water intake were recorded by weighing the bottles every other day. Bottle positions were interchanged every other day to control for development of position preference.

Two hours prior to sacrifice, ethanol was removed from the cages. In a preliminary study from our laboratory, trunk blood was analyzed for the presence of alcohol. At this time-point, the level of alcohol present in the blood was not pharmacologically relevant. In addition, upon observation, rats did not exhibit physical symptoms of withdrawal as outlined in Waller et al. (1982). At least one ethanol and one control animal were processed during the same experimental run. Rats were sacrificed by decapitation; the brain was removed and placed on a McIlwain tissue chopper with the rostral pole against the blade. Operating the tissue chopper in the manual mode, A 1.6 mm thick coronal section was taken at the level of the Acb (approximately 2.2 mm rostral to bregma) and placed on a microscope slide. Under a dissecting microscope, the frontoparietal cortex was trimmed from the section leaving the primary olfactory cortex intact. To obtain the Acb, a diagonal cut was made connecting the lateral ventricle to the primary olfactory cortex passing through the anterior commissure. A second cut was made from the ventral aspect of the lateral ventricle to the ventral surface of the slice. The resulting sample, referred to as the Acb, contains a small portion of the olfactory tubercle (Paxinos & Watson, 1982). This procedure was performed for both hemispheres. The tissue was weighed and homogenized in 50 volumes of 0.32 M sucrose. Samples of the homogenate were added to tubes containing Krebs Ringer Buffer and approximately 8nM [³H]DA. Samples were incubated in a water bath at 37°C for 2 minutes. Following incubation, the samples were centrifuged at 5500 × g for 15 minutes. Subsequently, supernatants were aspirated, and the pellets were surface-washed with 2 ml of ice cold saline. Following aspiration of the saline,

the tubes were inverted to drain. Tissue radioactivity was extracted by the addition of 0.5 ml 0.1 M NaOH, and following a 30 min period, samples were transferred to scintillation vials with 5 mls of Ecolite scintillation cocktail. Radioactivity was determined by liquid scintillation spectrometry.

For the kinetic analyses, two additional groups (water and ethanol-drinking) of female HAD-1 rats were utilized ($n = 9$ per group). The experimental procedures remained unchanged except that the tissue was homogenized in 70 volumes of 0.32 M sucrose and the assays were conducted in the presence of 5 concentrations of [^3H]DA ranging from 8–500 nM. For all uptake assays, nonspecific uptake was defined as the amount of uptake occurring at 4°C, and net DA uptake was determined by subtracting this uptake from that occurring at 37°C.

2.3. Statistical analyses

Data obtained from uptake experiments were converted to pmol DA uptake/mg tissue/2 minutes. Data were then analyzed by a one-way analysis of variance to determine differences in DA uptake between the chronically exposed EtOH and control groups. K_m and V_{max} were determined by plotting the saturation data as Eadie-Hofstee plots of velocity (v) against velocity/substrate concentration (v/s). In this type of plot, the y- and x-intercepts provide estimates of the V_{max} and K_m/V_{max} respectively, and the slope of the regression line provides an estimate of $-K_m$. Group differences were then analyzed using a one-way ANOVA to determine differences in the K_m and V_{max} between the groups.

3. Results

The average ethanol intake for the week prior to the sacrifice was 6.4 ± 0.3 g/kg/day (data not shown). Past studies have shown that ethanol intake in this range can yield pharmacologically relevant blood ethanol levels (c.f. Rodd et al., 2004). This amount of ethanol consumption significantly altered DA uptake in female HAD-1 rats. The analysis revealed that there was a significant effect [$F(1,7) = 17.48, p < 0.01$] of ethanol on DA uptake in the Acb compared to ethanol-naïve controls (Fig. 1). Rats exposed to a chronic regimen of 15% v/v ethanol exhibited a significant increase in DA uptake [Mean = 134 ± 9.4 fmols/mg tissue/2 minutes] in the Acb compared to ethanol-naïve rats [Mean = 74.4 ± 13.5 fmols/mg tissue/2 minutes]. This represents an increase in DA transport velocity of approximately 80 percent following chronic ethanol consumption.

Analyses of the kinetic data revealed a significant difference [$F(1,13) = 4.46, p = 0.05$] in the V_{max} of transport in rats chronically consuming 15% v/v ethanol [Mean $V_{max} = 8.48 \pm 1.2$ pmols/mg tissue/2 minutes] compared to water controls [mean $V_{max} = 5.59 \pm 0.9$ pmols/mg tissue/2 minutes]. Figure 2a depicts a representative saturation curve for the water control group. The corresponding representative Eadie-Hofstee plot is shown in Figure 2b. Figures 3a and b show a representative saturation curve and the corresponding representative Eadie-Hofstee plot, respectively, for the ethanol group. Further analyses revealed a statistically significant difference [$F(1,13) = 5.03, p < 0.05$] in the K_m in the ethanol-exposed animals [mean $K_m = 211.6 \pm 40.8$ nM] compared to controls [mean $K_m = 130.8 \pm 26.8$ nM].

4. Discussion

In the current study, chronic ethanol consumption increased the rate of accumulation of DA in the Acb homogenate. Following the initial experiment, a kinetic study was conducted to determine whether the increase in uptake was the result of a change in the maximum velocity of transport or a change in K_m or both. Chronic ethanol consumption resulted in a statistically significant increase in the V_{max} of DA transport in the nucleus accumbens of female HAD-1

rats. In addition, chronic ethanol consumption increased the K_m , which translates into a decrease in dopamine's affinity for the transporter.

The increase in ethanol-induced V_{max} could be the result of an increase in transporter density, a change in the rate at which the DAT traverses the membrane or both. However, the majority of the studies in the literature suggest that the increase in V_{max} is the result of an increase in transporter density. Drugs of abuse, such as cocaine (Itzhak and Martin, 1999; Letchworth et al., 2001) and ethanol (Itzhak and Martin, 1999; Jiao et al., 2006), have been shown to increase DA transporter binding sites. For example, Letchworth et al. (2001) demonstrated an increase in striatal DAT binding following chronic (approximately 3 months) treatment with cocaine. Binding in the striatum increased 21–42% following administration of the highest dose (0.3 mg/kg) of cocaine. Chronic ethanol consumption increased DAT binding sites in Wistar-Kyoto rats in several brain regions, including the Acb, compared to Wistar rats (Jiao et al., 2006). In addition, Itzhak and Martin (1999) reported an increase in striatal DAT density in Swiss Webster mice following administration of either cocaine or ethanol. It is possible, however, that the number of transporters remained the same, or even decreased, and the turnover rate increased considerably.

Few studies have examined both transporter function and density. DAT activity has been shown to obey Michaelis-Menten kinetics *in vitro* (Nicholson, 1995) and *in vivo* (Zahniser et al., 1999). Zahniser et al. (1999) reported an increase in DAT velocity as the dorsal striatal extracellular DA levels increased. Mayfield et al. (2001) examined the effects of ethanol on several dopaminergic parameters in *Xenopus* oocytes in which the human DAT had been expressed. Ethanol (10–100 mM) not only significantly increased DA accumulation in the oocytes but it also significantly increased DAT-mediated currents. A subsequent binding assay revealed an increase in cell surface DAT binding that paralleled the increase in DA uptake (Mayfield et al., 2001).

Ethanol has been shown to increase DA release resulting in higher concentrations of extracellular DA (Di Chiara and Imperato, 1985; Imperato and Di Chiara, 1986; Melendez et al., 2002; Weiss et al., 1993; Yim and Gonzales, 2000; Yoshimoto et al., 1991). The current results indicate that chronic ethanol consumption resulted in significant changes in DA transport. These alterations could reflect an increase in DA release. As a consequence, the activity of the transporter may have increased to compensate for the increased extracellular levels of DA. Another possibility is that transporter density has increased. Chronic ethanol exposure has been shown to increase the number of DA transporters in the nucleus accumbens (Mash et al., 1996) of monkeys.

In addition to the increase in V_{max} , an increase in the K_m was also noted. Thus, the higher K_m value in the ethanol group suggests a lower affinity for the transporter. However, it should be noted that apparent alterations in K_m can result from changes in endogenous substrate concentration in the incubation medium. Higher concentrations of endogenous DA could lead to an artifactual increase in the K_m , if not accounted for in the calculations of specific radioactivity. In the current study, no attempt was made to correct for possible differences in endogenous dopamine between the control group and the group consuming chronic ethanol. Based on the concept of simple competitive inhibition by endogenous dopamine, and the Michaelis-Menten equation for such inhibition, tissue from the chronic ethanol group would have to contribute approximately 80 nM to the incubation medium in order to account for the change in K_m from 131 nM (control) to 212 nM (chronic ethanol). Based on a tissue content for dopamine in the nucleus accumbens of HAD rats of approximately 50 pmols/mg tissue (Gongwer et al., 1989) and the inclusion of 2 mg of tissue/ml in the present uptake experiments, an estimated 80 percent of the endogenous dopamine would have to exit the tissue to account

for the observed change in K_m . It thus seems unlikely that the alterations in K_m resulting from chronic ethanol consumption would be explained by interference from endogenous dopamine.

It should be noted that the present studies were conducted in female rats. Microdialysis studies (e.g. Campbell et al., 1996) indicate no effects of the estrous cycle on ethanol-stimulated DA release. Walker et al. (2006) however, have reported gender differences when investigating the effects of dopaminergic drugs on DAT and autoreceptor function in the striatum of anesthetized rats. These authors, however, did not investigate the effects of ethanol. In a previous study, Walker et al. (2002) demonstrated an increase in the maximal velocity of DA uptake in female rats using fast cyclic voltammetry, but no gender differences in dopamine's affinity for the transporter. The authors also report that females exhibited greater extracellular dopamine following stimulation at 60 Hz than did males, but at lower levels of stimulation (i.e. 20Hz), there were no appreciable differences in extracellular DA levels. Thus, it is not surprising that the DA uptake rates were higher in the females, since uptake velocity is a function of substrate (DA) concentration. Nevertheless, in the future, it would be worthwhile to conduct a similar study in male rats.

Overall, the findings presented in the current study indicate that chronic ethanol consumption alters the function of the DAT in the HAD-1 rat line. Future studies will determine whether the effects of EtOH consumption on the DAT occurs in other rat lines/stocks and if the effect is persistent during a period of EtOH abstinence. The current results add to the growing literature indicating that EtOH consumption produces neuroadaptations in the mesolimbic DA system. The possibilities that these neuroadaptations are the biological basis for the progressive alteration in ethanol consumption from moderate to excessive levels need to be further explored.

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Figure 1.

Effect of chronic alcohol consumption on dopamine uptake in nucleus accumbens. Dopamine uptake was determined in homogenates in the presence of 8 nM dopamine. Data were analyzed using a one-way analysis of variance. Chronic ethanol consumption increased dopamine transport velocity approximately 80% compared to water controls [$F(1,7) = 17.48$, $p < 0.01$]. Data represent the mean $\pm$ standard error of the mean of 4 rats. The asterisk represents a significant effect of ethanol treatment.

Figure 2a,b.

Representative saturation curve (a) and the corresponding representative Eadie-Hofstee plot (b) for the water control group. Dopamine uptake was determined using dopamine concentrations ranging from 8–500 nM. All data were analyzed by using a one-way analysis of variance. The mean $V_{\max}$ of transport for the water group was 5.59 ± 0.9 pmols/mg tissue/ 2 minute (n=9); and the mean K_m for the water group was 130.8 ± 26.8 nM (n=9).

Figure 3a,b.

Representative saturation curve (a) and the corresponding representative Eadie-Hofstee plot (b) for the ethanol-drinking group. Dopamine uptake was determined using dopamine concentrations ranging from 8–500 nM. All data were analyzed by using a one-way analysis of variance. The mean $V_{\max}$ of transport for the ethanol group was 8.48 ± 1.2 pmols/mg tissue/2 minutes ($n=7$); and the mean K_m for the ethanol group was 211.6 ± 40.8 nM ($n=7$). Chronic ethanol consumption resulted in an increase in the $V_{\max}$ [$F(1,13) = 4.46$, $p < 0.054$] and K_m [$F(1,13) = 5.03$, $p < 0.05$] of transport compared to water controls.