Ethanol Exposure Differentially Alters Central Monoamine Neurotransmission in Alcohol-Preferring versus -Nonpreferring Rats

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Accepted for publication October 22, 1998 This paper is available online at http://www.jpet.org

ABSTRACT
Individual differences in ethanol preference may be linked to differences in the functional activity of forebrain monoamine systems or their sensitivity to modification by ethanol. To test this hypothesis, basal extracellular concentrations of dopamine (DA) and serotonin (5-HT) in the nucleus accumbens as well as the effects of repeated ethanol pretreatment on the basal release of these transmitters were examined in alcohol-preferring (P), alcohol-nonpreferring (NP), and genetically heterogeneous Wistar rats. All animals received i.p. injections of ethanol (1.0 g/kg) or saline for 5 consecutive days. Fifteen hours after the final pretreatment, basal extracellular concentrations and “in vivo extraction fraction” values for DA and 5-HT were determined by no-net-flux in vivo microdialysis. In ethanol-naive rats, significant line differences were observed with high basal 5-HT release in P rats, low 5-HT release in NP rats, and intermediate 5-HT levels in Wistar rats. No differences among groups were noted in basal DA release. Ethanol pretreatment decreased basal extracellular 5-HT levels in P rats whereas increasing 5-HT efflux was seen in the Wistar and NP lines. In addition, ethanol pretreatment increased extracellular DA concentrations in Wistar and P rats, but not in NP rats. The results confirm a relationship between the functional status of forebrain DA and 5-HT systems and ethanol preference or aversion. Moreover, the data suggest that ethanol exposure can alter basal DA and 5-HT in the nucleus accumbens and that vulnerability to ethanol-induced changes in monoamine neurotransmission may be a factor in genetically determined ethanol preference.

Genetic predisposition is an important factor in the propensity to develop alcoholism or increased sensitivity to the effects of alcohol (Schuckit et al., 1972; Cotton, 1979; Cloninger et al., 1981; Kiiannaa et al., 1992). The use of animals selectively bred to prefer or to avoid alcohol has been highly instrumental in advancing the understanding of the neurobiological basis of ethanol preference. In particular, this research has shown that abnormalities in neurotransmitter and receptor systems exist in animals that exhibit heightened ethanol preference and that these abnormalities may underlie the genetic susceptibility to ethanol abuse (for review, see McBride et al., 1990, 1991; Froehlich and Li, 1993; Li et al., 1994).

The majority of these investigations have focused on brain dopamine (DA) and serotonin (5-hydroxytryptamine, or 5-HT) systems. Many genetically selected rodent lines with divergent alcohol drinking behavior differ in forebrain dopaminergic and serotonergic function (Murphy et al., 1982, 1987; Yoshimoto and Komura, 1987; Gongwer et al., 1989; George et al., 1995). For example, the tissue content of 5-HT and its metabolite 5-hydroxyindoleacetic acid in various forebrain regions is substantially lower in alcohol-preferring rats such as the Indiana alcohol-preferring (P) and high alcohol-drinking (HAD) lines than in their alcohol-nonpreferring (NP) counterparts (Murphy et al., 1982, 1987; Gongwer et al., 1989). P rats also have a lower density of 5-HT neurons in the dorsal raphe nucleus (Zhou et al., 1991) and reduced serotonergic innervation of several 5-HT terminal regions (Zhou et al., 1991). Differences in serotonergic function between preferring and nonpreferring lines extend to the receptor level and include higher 5-HT1A binding (McBride et al., 1994) and lower 5-HT2 binding (McBride et al., 1993b) in P as opposed to NP rats.

P and NP rats also show distinct differences with regard to brain DA transmission. Lower forebrain tissue contents of DA with particularly marked deficits in the nucleus accumbens (NAC) have been documented in P (Murphy et al., 1982, 1987) and HAD rats (Gongwer et al., 1989). In addition, the Indiana P as well as the Sardinian preferring lines show reduced forebrain densities of D2 (Stefanini et al., 1992, McBride, 1993a) and D1 receptors (De Montis et al., 1993). In spite of these DAergic deficits, P rats show a heightened sensitivity to the DA release-enhancing or locomotor-activating effects of ethanol (Waller et al., 1986; Weiss et al., 1993).

Although these findings strongly implicate dysfunctions in...
forebrain DA and 5-HT neurotransmission as a factor in ethanol preference, several issues remain unresolved. First, to date, evidence on monoaminergic differences between high and low P rats is based largely on ex vivo, whole-tissue measures of DA and 5-HT rather than extracellular measures of transmitter release. In light of evidence that changes in tissue neurotransmitter content are not necessarily paralleled by corresponding changes at the synaptic level (Robinson and Whishaw, 1988; Robinson et al., 1990; e.g., Parsons et al., 1991a) it will be important to examine neurochemical differences between high and low P animals at the extracellular level. Second, there is evidence that repeated self-administration of ethanol (Weiss et al., 1996) or cocaine (Weiss et al., 1992; Parsons et al., 1995) can lead to persistent increases in basal DA release in the NAC of rats. Thus, it is possible that ethanol preference in genetically susceptible animals is linked not only to a "premorbid" dysfunction in DA or 5-HT transmission, but also to changes in these neurochemical systems that are induced by exposure to ethanol. Using rats of the behaviorally and neurochemically well characterized Indiana P and NP lines, the present study sought to identify differences in DA and 5-HT neurotransmission between P and NP rats at the extracellular level, and to examine whether repeated exposure to ethanol produces differential changes in DA and 5-HT release in P versus NP rats. Additionally, it was of interest to determine the direction of changes in DA and 5-HT function in P and NP rats with respect to a sample of genetically unselected Wistar rats (i.e., the progenitor line from which P and NP rats were originally derived). The measures taken were extracellular neurotransmitter levels and in vivo extraction fraction which has been shown to reflect changes in reuptake (Smith and Justice, 1993; Olson-Cosford et al., 1996).

Materials and Methods

Subjects. Male P and NP rats (Indiana University, Indianapolis, IN) as well as male Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) weighing 300 to 350 g at the time of testing were used. The animals were group-housed in a temperature- and humidity-controlled vivarium and maintained on a 12-h light/dark cycle with food and water available ad libitum. All experimental and surgical procedures were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Stereotaxic Surgery. Animals were anesthetized with a halothane/oxygen-carbon dioxide (95% O₂:5% CO₂) mixture and placed in a stereotaxic apparatus where they were implanted with a 20-µm microdialysis guide cannula (Plastics One, Roanoke, VA) aimed at the NAC. With respect to the bregma, the final coordinates for the placement of the guide cannula were AP -1.7, ML -1.5, and DV -6.1 (from dura), according to the atlas of Paxinos and Watson (1986). The guide cannula was secured to the skull with stainless steel screws and cranioplastic cement (Plastics One). Because of the severe hemorrhaging tendency of the NP rats, epinephrine was applied topically to the skull surface and exposed tissue of these animals during surgery to inhibit bleeding. NP rats were also given an injection of lactated Ringer's solution (1 ml/kg, s.c.) to replenish fluid loss during surgery. After surgery, all rats were given a 4- to 5-day recovery period before the beginning of the experiment.

Experimental Protocols and Design. After completion of the recovery period, rats of the P, NP, and Wistar groups were randomly assigned to one of two groups and injected i.p. once per day with either ethanol (1 g/kg; dissolved in 1 ml of saline) or saline (1 ml/kg) for 5 consecutive days. Thirty minutes after each injection, tail blood was obtained from all ethanol-treated animals to determine blood alcohol levels (BALs). To control for possible effects of stress associated with the bleeding procedure, the tails of saline-treated rats were also cut, but no blood was withdrawn. Blood samples were assayed for ethanol content by the NAD-NADH spectrophotometric method (Sigma Chemical Co., St. Louis, MO). Approximately 15 h after the final ethanol or saline injection, extracellular levels and in vivo extraction fractions of DA and 5-HT were determined by quantitative microdialysis as described below.

Intracranial Microdialysis. Microdialysis probes were constructed as described previously (Smith and Justice, 1993). Briefly, two lengths of fused silica (100 µm o.d. 40 µm i.d.) were inserted into a 5-mm section of cellulose dialysis tubing (200 µm; 6000 molecular weight cutoff, Spectrum Medical Industries, Houston, TX). Polyimide resin (Alltech Associates, Waukegon, IL) was used to seal both ends of the dialysis membrane. The inlet silica line extended beyond the outlet silica line by 2 mm inside the dialysis membrane to define the active area of the probe. The remaining portions of the dialysis membrane were coated with polyimide resin.

On the last day of the ethanol or saline pretreatment phase, microdialysis probes were inserted and rats were placed in the test chambers. The inlet line of the dialysis probe was connected to a 1-ml Hamilton syringe mounted on a CMA 100 syringe pump and perfusion was begun at 0.5 µl/min with artificial cerebrospinal fluid (aCSF) containing 149 mM NaCl, 2.8 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 0.25 mM ascorbic acid, and 5.4 mM d-glucose, pH 7.2 to 7.4. Approximately 15 h later, three baseline samples were collected at 15-min intervals. At this time, the no-net-flux procedure began by changing the perfusate to either aCSF containing DA (5, 10, or 20 nM) or 5-HT (5 or 10 nM). Five samples at 15-min intervals were collected at each concentration of DA and 5-HT. All concentrations for one neurotransmitter were completed before the no-net-flux procedure for the next neurotransmitter was begun. The order of the concentrations for a given neurotransmitter was randomized and the order of neurotransmitter was also randomized. After the no-net-flux experiment was completed for both neurotransmitters, the perfusate was changed to aCSF and three baseline samples were taken.

Analytical Procedures. Dialysate DA and 5-HT concentrations were determined by microbore reversed-phase HPLC. Samples were injected in 5-µl volumes via a VALCO high-pressure valve onto a 3-µm, C₁₈ column (1.0 × 100 mm, Sepstik; Bioanalytical Systems, Inc., West Lafayette, IN) which separated DA and 5-HT in 6 and 12 min, respectively. The mobile phase contained 19 mM citric acid, 21 mM Na₂HPO₄, 0.2 mM Na₂EDTA, 5 mM triethylamine, 0.25 mM d-decanesulfonic acid, and 20% methanol (apparent pH, 5.25) at a flow rate of 25 µl/min with an Isco LC-500 high-pressure syringe pump. The analytes were detected amperometrically by a BAS LC-4B detector (Bioanalytical Systems) with an applied potential of 700 mV versus Ag/AgCl. Calibration curves were constructed from DA and 5-HT standard solutions. Limits of detection were 0.1 nM for DA and 0.2 nM for 5-HT.

Data Analysis. Extracellular concentrations and in vivo extraction fraction for DA and 5-HT were obtained by linear regression. Regression lines were constructed by plotting the difference between the inflow and outflow concentrations of DA or 5-HT versus their inflow concentrations. The in vivo extraction fraction was derived from the slope of the regression line. The extracellular concentration (zero-point intercept) was obtained as \(-b/m\) by solving \(y = mx + b\) for \(x\) when \(y = 0\), where \(b\) is the y-intercept and \(m\) is the slope of the regression. The correlation coefficient of the regression was reported as Pearson's \(r\). Differences in the effects of ethanol and saline pretreatments on extracellular concentrations and in vivo extraction fractions were separately analyzed for DA and 5HT content by two-way ANOVA. After confirmation of significant effects in the overall ANOVA differences within each line of rats were determined by analysis of simple effects. Differences between the three lines were separately analyzed in the saline- and ethanol-pretreated groups by one-way ANOVA followed by Neuman-Keuls post hoc tests.
Histology. At the end of the experiment, animals were sacrificed by administering an overdose of halothane and the brains were removed and stored in 10% Formalin. To verify microdialysis probe placements, the brain was frozen on a cryostat and sliced into 30-μm coronal sections that were stained with cresyl violet. Slices were microscopically examined for correct placement using the atlas of Paxinos and Watson (1986).

Results

Histological analysis of coronal slices indicated that NP, P and Wistar rats’ probes were located within the same region of the NAC (Fig. 1).

Line Differences in Basal Extracellular Monoamine Levels. The mean (± S.E.M.) extracellular concentration and in vivo extraction fraction of DA in ethanol-naive (i.e., saline-pretreated) rats were 3.8 ± 0.7 nM and 40 ± 6% (NP, n = 5), 4.8 ± 0.8 nM and 40 ± 3.8% (P, n = 5), and 5.6 ± 0.4 nM and 50 ± 3% (Wistar, n = 5). No statistical differences in extracellular concentration (F(2,12) = 2.606, not significant; Figs. 2 and 3) or in vivo extraction fraction (F(2,12) = 1.534, not significant; Fig. 3) of DA were found among the three lines.

In contrast, a significant effect of line was obtained with respect to the extracellular concentration of 5-HT (F(2,14) = 20.031, p < .0001; Figs. 2 and 4). Ethanol-naive P rats had a mean (± S.E.M.) extracellular 5-HT concentration of 1.6 ± 0.2 nM (n = 5), which was significantly higher than in NP (0.4 ± 0.07 nM, n = 5, p < .01) and Wistar rats (0.7 ± 0.06 nM, n = 5, p < .01). Although ethanol-naive P rats showed elevated extracellular 5-HT levels, the in vivo extraction fraction of 5-HT in these animals (38 ± 3.3%) was significantly reduced (F(2,14) = 5.011, p < .05; Fig. 3) compared with both the NP (49 ± 2%; p < .05) and Wistar groups (47.8 ± 1.1% p < .05).

Effects of Ethanol Pretreatment. The BALs for the NP, P, and Wistar lines were 99.6 ± 4.1 mg% (n = 5), 102 ± 4 mg% (n = 5), and 100.6 ± 7.1 mg% (n = 5) respectively. There was no difference in BALs among the three groups. Five days of repeated treatment with ethanol resulted in a significant increase in basal extracellular DA concentrations in Wistar and P rats over the levels in their ethanol-naive counterparts (F(2,14) = 5.111, p < .05; Figs. 2 and 3). Mean (± S.E.M.) DA concentrations in Wistar rats increased from 5.6 ± 0.4 nM (n = 5; ethanol naive) to 8.2 ± 0.3 nM (n = 5; ethanol exposed; p < .05) and, in the P line, increased from 4.8 ± 0.8 nM to 7.9 ± 0.8 nM (p < .01; Figs. 2 and 3).

With respect to postethanol changes in extracellular 5-HT concentrations, ANOVA revealed a significant interaction between lines and treatment conditions (F(2,14) = 41.747, p < .0001; Fig. 4). Specifically, relative to the corresponding saline-pretreated groups, both the Wistar (p < .001) and NP group (p < .001) showed increases in basal 5-HT levels after repeated ethanol treatment, whereas basal 5-HT levels decreased in P rats (p < .01). Ethanol pretreatment also altered the in vivo extraction fraction of 5-HT (Fig. 4) which was higher in both NP (49 ± 2% versus 60 ± 3%, p < .01) and P rats (38 ± 3% versus 47 ± 3.6%, p < .05) compared with the respective saline control groups. There was no change in the in vivo extraction fraction of 5-HT between ethanol-naive (47.8 ± 1%) and ethanol-exposed Wistar rats (51.2 ± 1.8%).

Line Differences in Ethanol Effects. Statistical comparison of basal extracellular DA levels among the three ethanol-exposed groups revealed a significant effect of line (F(2,12) = 27.195, p < .00001, Fig. 3), with significantly higher basal DA levels in Wistar (p < .01) and P (p < .01) compared with NP rats. Comparison of the effects of ethanol pretreatment on basal extracellular 5-HT levels also revealed a significant effect of line (F(2,14) = 15.742, p < .001; Fig 0.4) with significantly higher 5-HT levels in NP rats (p < .01) than P and Wistar rats. In addition, there was a significant effect of line on the in vivo extraction fraction of 5-HT (F(2,14) = 4.936, p < .05; Fig 4) with significantly higher values in NP rats than P rats (p < .05).

Discussion

The results confirm that P and NP rats show differences in dopaminergic and serotonergic neurotransmission within the NAC and that previous exposure to ethanol can alter the activity of these monoamines systems. The line differences in extracellular DA and 5-HT levels provide further support for a role of these transmitter systems in alcohol preference and heightened alcohol-seeking behavior.

The present data which revealed higher extracellular 5-HT concentrations in P than NP rats and no line differences in DA release in ethanol-naive rats differ from earlier findings obtained with brain tissue preparations from P and NP rats which have identified deficiencies in accumalal whole-tissue content of both 5-HT and DA in P compared with NP rats (Murphy et al., 1982, 1987). Whole-tissue deficits in forebrain DA and 5-HT content have also been demonstrated in other genetically selected alcohol-preferring lines, such as the HAD versus low alcohol-drinking rats (Gongwe et al., 1989) as well as the C57BL/6J versus the DBA/2J and BALB/c mouse lines (George et al., 1995).

Although the previous tissue and present extracellular
Data seem paradoxical, they are not irreconcilable. First, it is well established that measures of neurotransmitter tissue content are not necessarily representative of transmitter levels in the extracellular space. For example, evidence for an uncoupling between tissue and extracellular neurotransmitter concentrations comes from observations that 6-hydroxydopamine lesions of the NAC which produce a substantial depletion of DA as measured by whole-tissue assays do not alter the extracellular levels of DA unless the depletion is severe (>90%) (Robinson and Whishaw, 1988; Robinson et al., 1990; Parsons et al., 1991a).

It is also possible, at least in the case of 5-HT, that the elevation of extracellular 5-HT levels in P rats may reflect some adaptive up-regulation of release to compensate for deficiencies in serotonergic innervation shown to exist in various 5-HT terminal regions (Zhou et al., 1991). In addition, as suggested by the lower in vivo extraction fraction of 5-HT in ethanol-naive P rats, these animals may have a lower rate of 5-HT reuptake. The in vivo extraction fraction in microdialysis experiment depends predominantly on changes in reuptake mechanisms and not neuronal release or metabolism (Smith and Justice, 1993; Olson-Cosford et al., 1996). Therefore, lower rates of 5-HT reuptake may be responsible for the elevated extracellular 5-HT levels in the P line. Finally, in at least one study that measured tissue contents of DA and 5-HT, the rats had undergone preference testing for ethanol and, thus, had significant exposure to ethanol before the neurochemical tests (Murphy et al., 1987).

Repeated exposure to drugs of abuse, including cocaine (Parsons et al., 1991b; Weiss et al., 1992; Parsons et al., 1995) and ethanol (Kaneyuki et al., 1991; Weiss et al., 1996), has been reported to cause profound alterations in basal DA and 5-HT neurotransmission. Thus, exposure to ethanol during preference testing may have altered the functional activity and possibly intraneuronal concentrations of DA or 5-HT, contributing to the discrepancies between the extracellular and earlier whole-tissue measures.

The hypothesis that previous exposure to ethanol may alter the functional status of forebrain monoamine systems was, in fact, supported by the present data. It is particularly intriguing with regard to a neurochemical hypothesis of ethanol preference that ethanol exposure produced a differential pattern of changes in P and NP rats. Repeated ethanol pretreatment with a dose of ethanol that produces BALs similar...
to those typically attained by rats in 30-min ethanol self-administration sessions increased basal extracellular concentrations of 5-HT in NP rats and decreased 5-HT efflux in P rats such that in ethanol-exposed rats, line differences between P and NP rats at the extracellular level paralleled the lower whole tissue 5-HT level obtained in P rats versus NP rats. Interestingly, the up-regulation of basal 5-HT release in the NP rats as a result of previous ethanol exposure is consistent with ethanol nonpreference because increased synaptic availability of 5-HT is typically associated with a suppression of voluntary ethanol intake (for review, see Sellers et al., 1992; LeMarquand et al., 1994). Thus, the respective increases and decreases in extracellular 5-HT levels in NP and P rats after repeated exposure to ethanol may contribute to the development and maintenance of ethanol preference versus nonpreference. This possibility is supported by the good correspondence between the rank order of extracellular 5-HT levels and the rank order of ethanol preference and voluntary self-administration among the three lines of rats. Only P rats show spontaneous ethanol preference and although Wistar and NP rats can be trained to self-administer ethanol, they consume less ethanol than P rats with lower rates of intake in NP than in Wistar rats (Weiss et al., 1990; Files et al., 1993; Rassnick et al., 1993). Given that the degree of change in 5-HT levels induced by repeated ethanol was greatest in NP rats it is possible that elevated extracellular 5-HT levels play a particularly important role in ethanol nonpreference.

In the case of DA, repeated ethanol administration resulted in increased basal extracellular concentrations in P rats and no changes in NP rats. Although these observations remain inconsistent with the intraneuronal DA deficits in previous whole-tissue assays, they are consistent with other findings that intermittent exposure to ethanol (Kaneyuki et al., 1991, Weiss et al., 1996) and cocaine (Weiss et al., 1992; Parsons et al., 1995) can elevate basal DA concentrations at the extracellular level. The significance of this up-regulation in DA release after intermittent exposure to drug and alcohol-seeking behavior is as yet unclear. But it is possible that, like the ethanol-induced suppression of basal 5-HT release, this change plays a role in the “acclimation” to ethanol and, perhaps, the development of ethanol preference. Specifically, the absence of any differences in basal DA concentrations between P and NP rats before ethanol exposure in conjunction with the differential changes in the basal DA efflux after repeated ethanol treatment suggests that the heightened alcohol preference of the P rats may be driven by a greater sensitivity or increased vulnerability to ethanol-induced changes of the dopaminergic system in these rats. This interpretation is supported by previous reports of greater sensitivity to ethanol-induced DA release in alcohol self-administering P compared with Wistar rats (Weiss et al., 1993).
It was also of interest to gain insight into the nature and direction of changes in DA and 5-HT function that are associated with ethanol preference versus nonpreference in P and NP rats with respect to an unselected population of Wistar rats. In ethanol-naive animals, basal extracellular concentrations of 5-HT in the Wistar rats were comparable to those in NP rats but significantly lower than in P rats. After repeated ethanol administration, there was a significant increase in basal 5-HT concentrations in Wistar rats similar, albeit smaller in magnitude, than that in NP rats. Basal DA levels in ethanol-naive Wistar rats were statistically identical with those in P and NP rats. However, like in P rats, DA concentrations in Wistar rats increased in response to repeated ethanol exposure. Thus, in both ethanol-naive and ethanol-exposed Wistar rats, extracellular 5-HT concentrations were intermediate between those in NP and P rats, whereas with respect to DA the neurochemical profile of both ethanol-naive and ethanol-exposed Wistar rats was more similar to that in P than NP rats.

In conclusion, the extracellular concentration of 5-HT was higher in ethanol-naive P rats than in ethanol-naive NP and Wistar rats which may be the result of a reduced rate of reuptake in the P line. There was no difference in the extracellular concentration of DA among the three groups. In comparing the extracellular monoamine profile that emerged after ethanol exposure in Wistar versus P and NP rats, it appears that the serotonergic changes may be involved in the ethanol preference of P rats, but more importantly, in the ethanol nonpreference of NP rats. After repeated ethanol administration, the extracellular concentration of DA increased in P rats but not in NP rats, suggesting that differences exist between the two lines in the sensitivity of the dopaminergic system to ethanol, and a possible involvement of DA in ethanol preference. However, because the spontaneous ethanol preferring (P rats) and progenitor line (Wistar) showed similar increases in basal DA levels after repeated ethanol administration, these dopaminergic changes alone are unlikely to account for the ethanol preference of the P rats. Rather, the data suggest that both increased DA and reduced 5-HT transmission or an increase in the ratio of DA/5HT release is associated with ethanol preference because this ratio increased in P rats after ethanol exposure and decreased in Wistar and NP rats.

References


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