ELSEVIER

Pharmacology, Biochemistry and Behavior 71 (2002) 111–117

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Ethanol-stimulated serotonin release in the ventral hippocampus: an absence of rapid tolerance for the alcohol-preferring P rat and insensitivity in the alcohol-nonpreferring NP rat

R.J. Thielen[a,*], D.J. Bare[a], W.J. McBride[a,b], L. Lumeng[b,c,d], T.-K. Li[b,c]

[a]*Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202, USA*
[b]*Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, USA*
[c]*Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA*
[d]*Veterans Administration Medical Center, Indianapolis, IN 46202, USA*

Received 18 January 2001; received in revised form 9 July 2001; accepted 31 July 2001

Abstract

This study examined the acute effects of intraperitoneal administration of ethanol on the extracellular levels of serotonin (5-HT) in the ventral hippocampus (vHIP) of adult, male alcohol-preferring P and -nonpreferring NP rats. Using in vivo microdialysis coupled with HPLC and electrochemical detection, the effects of acute administration of saline or 1.0, 1.75, or 2.5 g/kg ethanol on the extracellular levels of 5-HT in the vHIP were examined. Saline and 1.0 g/kg ethanol did not alter the extracellular levels of 5-HT. However, the 1.75-g/kg dose resulted in a transient increase in 5-HT levels in the vHIP of P rats only. Administration of 2.5 g/kg ethanol increased 5-HT levels to 180% of baseline in P rats ($P < .05$), but was without effect on NP rats. The 2.5-g/kg dose also significantly increased the extracellular levels of 5-HT in the vHIP of P rats, which had been pretreated with the same dose of ethanol 18–24 h earlier ($P < .05$). Comparison of the response of ethanol pretreated P rats with animals that had been pretreated with saline 24 h earlier did not reveal any significant differences in ethanol-stimulated increases in 5-HT levels between the groups. These data suggest that ethanol may activate terminals of the median raphe 5-HT system in P rats because the vHIP receives its 5-HT inputs primarily from the median raphe nucleus (MRN). Rapid tolerance does not develop to this activation of the system in the vHIP of P rats. In addition, the data suggest that the 5-HT system in the vHIP of NP rats may be relatively insensitive to the stimulating effect of acute ethanol of 5-HT release.

Keywords: Median raphe nucleus; Ventral hippocampus; Acute ethanol; Serotonin; Microdialysis; Alcohol-preferring P rats; Alcohol-nonpreferring NP rats

1. Introduction

In vivo microdialysis studies have shown that acute intraperitoneal administration of ethanol increases the extracellular levels of serotonin (5-HT) in the nucleus accumbens of Wistar (Yoshimoto et al., 1992a) and the selectively bred high alcohol-drinking (HAD) and low alcohol-drinking (LAD) lines of rats (Yoshimoto et al., 1992b), and in the ventral hippocampus (vHIP) of Wistar rats (Bare et al., 1998). Additionally, in Wistar rats, rapid tolerance develops to the ethanol-stimulated increase in 5-HT levels in the vHIP (Bare et al., 1998), an area that receives 5-HT innervations primarily from the median raphe nucleus (MRN) (Azmitia and Segal, 1978).

Several studies have suggested a role for 5-HT in the development of tolerance to the motor-impairing and hypothermic effects of ethanol. Depletion of brain 5-HT (Frankel et al., 1978a,b) or lesions of the 5-HT system (Le et al., 1980) reduce the rate of tolerance development to hypothermic and motor-impairing effects of ethanol. Additionally, administration of the 5-HT precursor L-tryptophan increases the rate of tolerance development to these effects of ethanol (Le et al., 1979). The 5-HT projections from the MRN appear to be involved in mediating tolerance development to ethanol, in that electrolytic lesions of the MRN, but not the dorsal raphe nucleus (DRN), reduces the rate of tolerance development to the hypothermic and motor-impairing effects of ethanol (Le et al., 1981).

* Corresponding author. Institute of Psychiatric Research, Virginia Commonwealth University, 791 Union Drive, Indianapolis, IN 46202-4887, USA. Tel.: +1-317-274-2333; fax: +1-317-274-1365.
E-mail address: rthielen@iupui.edu (R.J. Thielen).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00633-5

Alcohol-preferring P and -nonpreferring NP rats have been selectively bred from an outbred Wistar stock for high and low alcohol-seeking behavior, respectively (Lumeng et al., 1977). In addition to high alcohol intake, P rats differ from alcohol-nonpreferring NP rats and Wistar rats in some behavioral responses to ethanol. P rats readily develop rapid tolerance to the high dose motor-impairing effects of ethanol and this tolerance persists for three to four times longer in P rats compared to NP and Wistar rats (Gatto et al., 1987). In addition, P rats demonstrate a number of differences in serotonergic measures compared to NP rats, including lower tissue levels of 5-HT in the hippocampus and several other regions (McBride et al., 1993; Murphy et al., 1982, 1987). The reduced levels of 5-HT in P rats appear to be a result of reduced serotonergic innervation (Zhou et al., 1991, 1994a) and fewer 5-HT neurons in the MRN and DRN (Zhou et al., 1994b). The reduced number of 5-HT neurons in the DRN did not result in a compensatory increase in firing rates of the remaining neurons because there was no difference in the firing rates of 5-HT neurons in the DRN between P rats and NP or Wistar rats (Morzorati and Johnson, 1999). However, Smith and Weiss (1999) have shown that P rats, compared to NP and Wistar rats, have higher basal extracellular levels of 5-HT in the nucleus accumbens, an area that receives 5-HT innervation primarily from the DRN (Azmitia and Segal, 1978; Steinbusch and Nieuwenhuys, 1983).

Because of the reduced 5-HT innervation in the hippocampus of the P rat (Zhou et al., 1991, 1994a), and the difference in the persistence of tolerance to the motor-impairing effects of ethanol observed for the P line compared to NP and Wistar rats (Gatto et al., 1987), the present study was undertaken to examine the effects of acute intraperitoneal ethanol administration on the response of MRN 5-HT terminals in the vHIP of P and NP rats. Currently, there are no data available on the effects of ethanol administration on components of the MRN 5-HT system of P and NP rats. Therefore, the present study was undertaken to examine the effects of acute ethanol administration on the extracellular levels of 5-HT in the vHIP of P and NP rats, and to determine if prior exposure to a high dose of ethanol alters the response of this 5-HT system to a subsequent ethanol challenge given 24 h later. The hypothesis to be tested is that the vHIP 5-HT systems of P and NP rats are sensitive to the acute effects of ethanol and that rapid tolerance will develop to the effects of ethanol on this 5-HT system in P but not NP rats.

2. Methods

2.1. Animals

Adult, male alcohol-preferring P and -nonpreferring NP rats from generations 43–48, weighing 250–350 g at the time of surgery, were housed individually in temperature- and humidity-controlled rooms on a normal 12-h light/dark cycle (lights on at 7:00 a.m.). Food and water were available ad libitum. The animals used in this experiment were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH, and the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

2.2. Stereotaxic surgery

Animals were habituated to the handling procedures necessary for intraperitoneal ethanol administration for 2 days prior to surgery. Animals were anesthetized with 2% isoflurane and, with the aid of a stereotaxic apparatus (David Kopf, Instruments, Tujanga, CA, USA), implanted unilaterally with a 21-gauge stainless guide cannula (Plastics One, Roanoke, VA, USA) aimed at the vHIP (using the following coordinates relative to bregma: rostral -5.6, lateral $+4.6$, and ventral -4.2 mm), according to the atlas of Paxinos and Watson (1998). The guide cannula was secured to the skull with Cranioplastic cement (Plastics One) anchored by two stainless steel screws. Animals were allowed to recover for at least 5 days before probes were inserted. During the recovery period, animals were habituated to the clear Plexiglas microdialysis chambers ($22.5 \times 44.5 \times 38.0$ cm, $W \times L \times H$) and the injection procedure for at least 2 days.

2.3. Microdialysis procedure

On the day before microdialysis between 9 and 11 a.m., animals were briefly anesthetized (less than 15 min) and a microdialysis probe was inserted. The concentric microdialysis probes were constructed as previously described (Parsons and Justice, 1992; Weiss et al., 1993), having a 2-mm active dialysis surface (Spectrum/Por 6 regenerated cellulose dialysis membrane, molecular weight cutoff 8000 Da; Medical Industries, Los Angeles, CA, USA) and extending 4 mm below the guide cannula, into the vHIP. After probe insertion, rats were placed into the microdialysis chambers. The animals were assigned to one of five groups with four of the groups being pretreated with saline (equivalent to the volume of intraperitoneal ethanol injections) 24-h prior to microdialysis, whereas the fifth group was administered 2.5 g/kg ethanol (see below). Animals were returned to their home cage 2 h later.

On the day of microdialysis (6 days postsurgery), animals were placed in the microdialysis chambers and the microdialysis probes were perfused with artificial cerebral spinal fluid (ACSF, composition, in mM: NaCl, 140; KCl 3.0; $CaCl_2$, 2.5; $MgCl_2$, 1.0; L-ascorbic acid, 0.2; adjusted to pH 7.2 with 2 mM Na_2HPO_4) at a flow rate of 0.32 μl/min using a microliter syringe pump (Harvard Apparatus, South

Natick, MA, USA). After a 2-h equilibration period, which allows 5-HT levels to stabilize, six baseline samples were collected every 25 min in microfuge tubes containing 2 μl of 0.2 mM EDTA, 0.33 mM L-cysteine, and 0.05 mM L-ascorbic acid in 0.1 N acetic acid. All samples were immediately frozen on dry ice and stored at −70 °C until assayed for 5-HT levels. During the experiment, animals were allowed to move freely throughout the microdialysis chamber. After the equilibration period, Groups 1–4, which had been injected with saline 24 h earlier, were administered either saline alone or 1.0, 1.75, or 2.5 g/kg ethanol (given as a 12% v/v solution), respectively, whereas Group 5, which had received 2.5 g/kg ethanol 24 h earlier, was administered a challenge dose of 2.5 g/kg ethanol. All ethanol solutions were given as a 12% v/v solution. Samples were collected for an additional 175 min.

2.4. Probe placements

At the end of the experiment, the probes were perfused with 1% bromophenol blue to mark the probe tip location. The animals were overdosed with CO_2, and the brains removed and frozen. Brain sections (40 μm) taken around the probe location were stained with Cresyl violet. Only animals with verified probe placements in the vHIP were included in the data analysis.

2.5. Determination of 5-HT levels in dialysates

Microdialysate samples were analyzed for 5-HT levels using a microbore HPLC with electrochemical detection. The samples were injected onto a reverse-phase microbore column (1.0 × 100 mm SepStik Spherisorb C18 column, 3 μm particle size, Bioanalytical Systems, West Lafayette, IN, USA) with a Rheodyne injector (5 μl loop, Cotati, CA, USA). The samples were separated using a mobile phase composed of 100 mM sodium acetate, 0.5 mM EDTA, 1.25 mM sodium octylsulfonic acid, 10 mM NaCl, and 10.5% acetonitrile, pH 5.0, with glacial acetic acid at a flow rate of 0.07 ml/min (Model 2350 pump, ISCO, Lincoln, NE, USA). The concentration of 5-HT was determined by electrochemical detection (+450 mV potential, 0.5 nA sensitivity, EG&G Princeton Applied Research, Princeton, NJ, USA) using a 6-mm radial-flow glassy-carbon electrode (Bioanalytical Systems). The injector, column, and electrode were contained in a UniJet CC-6 cabinet (Bioanalytical Systems), which allows for very low dead volume and lower detection limits. Output from the detector was sent to a microprocessor and integrated using ChromPerfect Spirit (version 4.4.21, Justice Innovations, Palo Alto, CA, USA) The detection limit for 5-HT was 20 pM.

2.6. Data analysis

Microdialysis time-course data are expressed as percent of baseline. The data were analyzed by mixed analysis of variance (ANOVA). Significant interactions were further analyzed by simple main effects. Where appropriate, Student's *t* tests were used to compare individual means.

3. Results

Representative probe placements for the animals used in this study are shown in Fig. 1. Only animals with verified probe placements in the vHIP were included in the data analysis. All were in the vHIP within approximately 1.5 mm of each other. Probes were placed between 4.52 and 6.04 mm caudal of bregma according to the atlas of Paxinos and Watson (1998).

Basal extracellular 5-HT levels did not differ between P ($n = 27$) and NP rats ($n = 28$) pretreated with saline (164 ± 24 and 119 ± 18 pM, respectively, $P = .14$). The basal levels in P and NP rats are lower than 300 pM basal levels of 5-HT previously reported in the vHIP for Wistar rats (Bare et al., 1998). However, it is not possible to make a meaningful comparison of the basal extracellular levels of 5-HT between P, NP, and Wistar rats without using the quantitative no net flux technique.

The effect of different doses of ethanol on extracellular 5-HT levels in the vHIP of P and NP rats is shown in Fig. 2. ANOVA of the time-course data revealed a significant Time × Dose × Line interaction [$F(21,329) = 1.66$, $P < .05$]. To explore the significant three-way interaction, the lines were analyzed separately. There was no effect of different doses of ethanol on extracellular 5-HT levels over time in the vHIP of NP rats [Time × Dose interaction, $F(21,168) = 1.19$, $P = .26$]. On the other hand, 5-HT levels were significantly

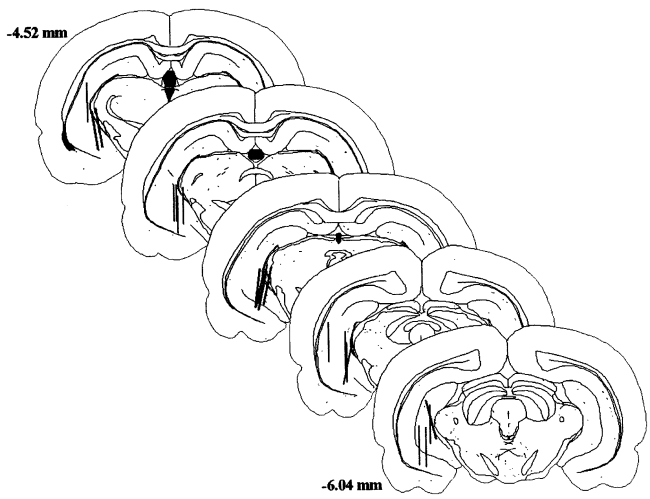

Fig. 1. Location of representative probe placements in the vHIP. Probe placements are shown on a diagram using the rostral/caudal coordinates relative to bregma as reported by Paxinos and Watson (1998). The thick lines represent the 2-mm active dialysis sites for each probe. For the most rostral and caudal sections, the distance from bregma is shown. Modified from Paxinos and Watson (1998).

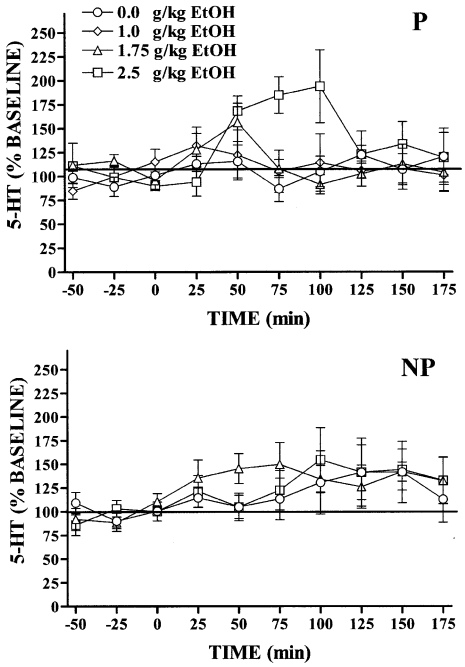

Fig. 2. The time-course effect of different doses of ethanol (EtOH) on the extracellular levels of 5-HT in the vHIP of P and NP rats. Baseline levels were determined by averaging the extracellular levels of 5-HT in the three samples immediately preceding EtOH or saline administration. After a stable baseline was established, each animal was given a single injection of saline (P, $n=9$; NP, $n=7$), 1.0 (P, $n=6$), 1.75 (P, $n=5$; NP, $n=9$), or 2.5 (P, $n=7$; NP, $n=8$) g/kg EtOH in saline. Data are expressed as percent of baseline (mean ± S.E.M.).

increased in the vHIP of P rats after ethanol administration as indicated by a significant Time × Dose interaction [$F(21,161)=2.13$, $P>.005$]. To further examine the Time × Dose interaction in the P rats, analysis of simple main effect of time was performed for each dose. This analysis revealed that saline and 1.0 g/kg ethanol administration did not significantly alter extracellular 5-HT levels in the vHIP of P rats ($P>.8$); however, administration of 1.75 and 2.5 g/kg ethanol induced a significant increase in extracellular 5-HT levels over baseline levels ($P<.05$).

The effect of a 2.5-g/kg ethanol challenge dose in animals given the same dose of ethanol 24 h earlier is shown in Fig. 3. The data for the saline–saline groups and the saline–ethanol groups are the same as those shown in Fig. 2 for the saline and 2.5-g/kg ethanol groups, respectively. The mean basal extracellular levels of 5-HT in the ethanol-pretreated P and NP rats were 180 ± 22 and 120 ± 17 pM, respectively, and did not differ significantly from the groups pretreated with saline [Line × Pretreatment interaction: $F(1,70)=0.074$, $P=.79$]. The ANOVA of the time-course data comparing both lines revealed a significant Time × Treatment × Line interaction [$F(14,308)=1.72$, $P=.05$]. The significant three-way interaction was explored further by analyzing the lines separately. As the NP rats did not demonstrate an increase in extracellular 5-HT levels in response to an acute administration of 2.5 g/kg ethanol, the pretreated animals were examined to determine if sensitization to ethanol-stimulated increases in 5-HT overflow occurs in NP rats. However, there was no significant effect of pretreatment on vHIP 5-HT extracellular levels after a 2.5-g/kg challenge dose of ethanol in NP rats [Time × Treatment interaction: $F(14,140)=0.55$, $P=.89$]. On the other hand, P rats had a significant Time × Treatment interaction [$F(14,168)=3.64$, $P<.001$]. The significant interaction in P rats was furthered explored by simple main effect analysis of time for each treatment group. As already noted, saline administration did not alter extracellular 5-HT levels in the vHIP but administration of 2.5 g/kg ethanol to ethanol-naive animals resulted in a significant increase in extracellular 5-HT levels (Fig. 2). Analysis of the ethanol-pretreated group revealed that the 2.5-g/kg ethanol challenge significantly increased extracellular 5-HT levels in the vHIP of P rats ($P<.001$) as it had in saline-pretreated P rats. In addition, the response of extracellular 5-HT levels in the vHIP of P rats was unaffected by ethanol pretreatment as indicated by comparison of individual means between the saline–ethanol and ethanol–ethanol groups with Student's t test at each time point that revealed that the two groups did not significantly differ in the magnitude of the increase in extracellular 5-HT levels in the vHIP at any time point ($P>.16$).

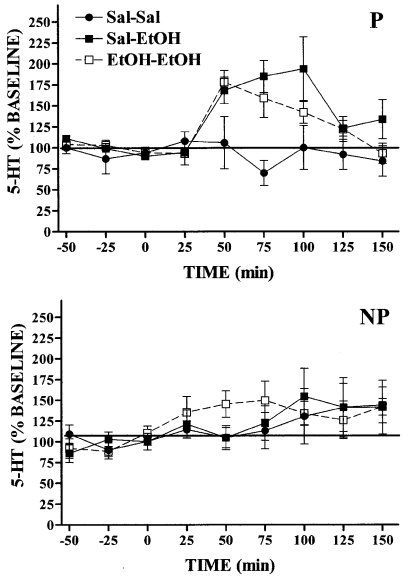

Fig. 3. The time-course effect of pretreatment with saline or 2.5 g/kg ethanol (EtOH) on the subsequent effect of a challenge dose of EtOH on extracellular levels of 5-HT in the vHIP of P and NP rats. Animals were pretreated with a single injection of saline (Sal–Sal and Sal–EtOH groups) or 2.5 g/kg ethanol (EtOH–EtOH) 18–24 h before the start of microdialysis. Baseline levels were determined by averaging the extracellular levels of 5-HT in the three samples immediately preceding EtOH or saline administration. After a stable baseline was established, each animal was given a single injection of saline (Sal–Sal group: P, $n=9$; NP, $n=7$) or 2.5-g/kg EtOH (Sal–EtOH group: P, $n=7$; NP, $n=8$, and EtOH–EtOH groups: P, $n=11$; NP, $n=8$). The data for the saline–saline group (Sal–Sal) and the saline–EtOH group (Sal–EtOH) are the same as those shown in Fig. 2 for the saline and 2.5-g/kg EtOH groups, respectively. Data are expressed as percent of baseline (mean ± S.E.M.).

4. Discussion

Results from this study suggest that ethanol activates the MRN terminals in the vHIP when moderate to high doses are administered to alcohol-preferring P rats but not to alcohol-nonpreferring NP rats, and that rapid tolerance does not develop to the ethanol-stimulated increase in extracellular 5-HT levels in the vHIP of P rats. These conclusions are supported by the findings that ethanol increased the extra-cellular 5-HT levels in the vHIP of P but not NP rats in a dose-related manner (Fig. 2). Additionally, these results suggest that rapid tolerance to this effect does not develop in the MRN system projecting to the vHIP of P rats because a challenge dose of ethanol elicited a similar increase in the extracellular levels of 5-HT in P rats pretreated 24 h earlier with either saline or ethanol (Fig. 3).

There were no significant differences in basal 5-HT extracellular levels in the vHIP of P and NP rats in this study, although P rats tended to have slightly higher basal 5-HT levels compared to NP rats. However, traditional microdialysis methods do not provide accurate measures of the true basal extracellular levels of neurotransmitters, whereas quantitative microdialysis techniques do provide accurate basal neurotransmitter levels (Justice, 1993; Parsons and Justice, 1994). The trend for higher basal 5-HT levels in the P rats compared to NP rats is in agreement with quantitative no net flux microdialysis data demonstrating higher basal 5-HT levels in the nucleus accumbens of P compared to NP rats (Smith and Weiss, 1999).

The moderate- to high-dose stimulating effect of ethanol on extracellular 5-HT levels in the vHIP of P rats is consistent with a number of studies showing an activating effect of ethanol on 5-HT systems. Previous studies demonstrated an increase in 5-hydroxyindoleacetic acid (5-HIAA) tissue levels in the striatum and nucleus accumbens 60 min after an acute administration of 2.5 g/kg ethanol to alcohol-naive Wistar rats (Khatib et al., 1988), as well as in P rats (Murphy et al., 1988). In addition, in vivo microdialysis studies have shown that acute administration of 2.0–2.5 g/kg ethanol to alcohol-naive rats increases extracellular levels of 5-HT in the nucleus accumbens (Yoshimoto et al., 1992a,b), anterior caudate–putamen (Thielen et al., 2001), and frontal cortex (Portas et al., 1994). Importantly, a previous study, in Wistar rats, demonstrated an activating effect of ethanol on 5-HT terminals in the vHIP, presumable of the MRN system (Bare et al., 1998). In that microdialysis study, doses of 1.75 and 2.5 g/kg ethanol also enhanced the extracellular levels of 5-HT in the vHIP (Bare et al., 1998) to approximately the same degree observed in the present study (Fig. 2). The nucleus accumbens, frontal cortex (Azmitia and Segal, 1978; Steinbusch and Nieuwenhuys, 1983), and striatum (Azmitia and Segal, 1978; Kreiss and Lucki, 1994; Steinbusch and Nieuwenhuys, 1983) receive their 5-HT projections primarily from DRN, whereas the vHIP receives its primary 5-HT input from the MRN (Azmitia and Segal, 1978; Kreiss and Lucki, 1994; Steinbusch and Nieuwenhuys, 1983). Therefore, the present findings suggest that the terminals of the ascending MRN 5-HT pathway may be activated by the ethanol, and that selective breeding for high alcohol consumption may not alter this initial response when compared with findings for Wistar rats.

In contrast to the results with P and Wistar rats (Bare et al., 1998), moderate to high doses of ethanol had no significant effect on extracellular 5-HT levels in the vHIP of NP rats. The lack of effect of ethanol on 5-HT overflow is not likely a result of different blood alcohol levels achieved after the intraperitoneal administration of ethanol as a number of studies have demonstrated similar BACs and ethanol elimination in P and NP rats after intraperitoneal ethanol administration. The BACs in P and NP rats, approximately 30 min after a 2.0-g/kg ethanol dose, were reported to be 250 ± 5 and 266 ± 8 mg%, respectively (Waller et al., 1983). Similarly, after the same dose of ethanol, Lumeng et al. (1982) reported that BACs taken from P and NP rats did not differ at 1, 2, 3, and 4 h after administration. More recently, Stewart et al. (1992) reported that BACs in P and NP rats 2 h after administration of 3.5 g/kg ethanol ip were 389 ± 8 and 389 ± 8 mg%, respectively. Therefore, in contrast to the findings with the P rats, it appears that selective breeding for low alcohol consumption may attenuate this initial response as compared with findings for P and Wistar rats.

The mechanism by which ethanol increases the extracellular 5-HT levels in the vHIP of P and Wistar rats is not defined; however, evidence suggests that ethanol probably increases the release of 5-HT rather than inhibiting its reuptake. If ethanol were inhibiting 5-HT reuptake, a decrease in 5-HIAA levels, as a result of reduced degradation of 5-HT, would be expected; however, ethanol administration does not result in reduced tissue content (Khatib et al., 1988; Murphy et al., 1988) or extracellular levels (Yoshimoto et al., 1992b) of 5-HIAA. Additionally, although the direct effect of ethanol on 5-HT transport has not been studied extensively, one study examining the effect of ethanol on 5-HT reuptake has demonstrated ethanol produces increases, rather than decreases, in 5-HT transport in synaptosomes (Alexi and Azmitia, 1991). Also, it is not clear whether ethanol is altering the release of 5-HT from terminals or increasing the firing rate of MRN 5-HT neurons. In support of the former hypothesis, results from in vivo electrophysiological studies in the DRN show a decreased firing rate of these neurons in response to ethanol administration (Thielen et al., 2001). However, conclusive studies examining the effect of ethanol on 5-HT neuronal activity within the MRN have not been conducted (Chu, 1984). A local effect of ethanol on 5-HT terminals is also supported by data showing that reverse dialysis of ethanol (100 mM) results in an increase in extracellular 5-HT levels in the nucleus accumbens (Yoshimoto et al., 1992a). Therefore, one possible action of ethanol in the vHIP of P rats may be to

increase the release of 5-HT through either a direct action on the 5-HT terminals or indirectly on inputs controlling 5-HT terminal release. Interestingly, the effect of ethanol on 5-HT release in the vHIP of P rats is not observed in the first 25-min collection period after ethanol administration but becomes significant in the second collection period. This is consistent with results in both the caudate–putamen and vHIP of Wistar rats where a similar delay was also seen after the 2.5-g/kg ethanol administration (Bare et al., 1998; Thielen et al., 2001). These results are consistent with ethanol acting through inputs onto 5-HT terminals and indirectly increasing 5-HT release.

Tolerance that develops 8–24 h after a single administration of ethanol has previously been defined as rapid tolerance (Crabbe et al., 1979; Kalant, 1993). It has been suggested that rapid tolerance may be an index of chronic tolerance and that rapid and chronic tolerance may be produced by similar mechanisms (Khanna et al., 1991, 1996). When challenged with a 2.5-g/kg dose of ethanol, P rats pretreated with this same dose of ethanol 24 h earlier showed a similar increase in extracellular 5-HT levels in the vHIP as P rats pretreated with saline (Fig. 3). Although it appears that the duration of the increase in 5-HT levels is shorter in the alcohol-pretreated compared to saline-pretreated P rats, there was no significant difference between these groups at any time point (Fig. 3). In addition, a previous study demonstrated that P rats do not develop rapid metabolic tolerance to high doses of ethanol. In this study, there was no difference in BACs in P rats 2 h after a 3.5-g/kg ethanol ip, given 24 h after an initial administration of the same dose compared to BACs at the same time point after the initial administration (BACs: Day 0, 389 ± 8; Day 1, 392 ± 5 mg%) (Stewart et al., 1992). These data suggest that rapid tolerance does not develop to the ethanol-stimulated increase in 5-HT overflow in the vHIP of P rats. These data are different than findings with Wistar rats, which suggested that rapid tolerance may develop to the ethanol-stimulated increase in 5-HT overflow, as measured by in vivo microdialysis, in the vHIP (Bare et al., 1998). This difference may arise from variations in the rate of rapid tolerance development in this neurochemical measure between Wistar and P rats, or possibly that rapid tolerance to ethanol-stimulated increases in extracellular levels of 5-HT does not occur in P rats. However, a previous study demonstrated that chronic tolerance might develop to the effects of ethanol on 5-HT release from DRN projections of P rats (Murphy et al., 1988). In that study, 5-HIAA tissue levels were increased following a challenge dose of 2.5 g/kg ethanol in the nucleus accumbens of alcohol-naive P rats but not in the nucleus accumbens of P rats that had developed tolerance to the motor-impairing effects of ethanol (Murphy et al., 1988). This suggests that repeated exposure to ethanol may be needed to produce tolerance to the effects of ethanol on the 5-HT system in P rats.

The present finding, indicating that rapid tolerance does not develop to the effects of ethanol-stimulated 5-HT release, suggests that this 5-HT system may not play a direct role in the development of rapid tolerance observed in the behavioral study (Gatto et al., 1987). This could mean that, in the P rat, other neuronal systems might play a role in the development of tolerance to compensate for the innate deficiencies in the 5-HT systems (Zhou et al., 1991, 199a,b). It is also possible that higher doses of ethanol may be required to observe the development of rapid tolerance in this 5-HT system. However, another possibility is that the different responses of the P and NP vHIP 5-HT systems to acute systemic ethanol administration may be related to differences in behavioral tolerance development seen between these lines.

In conclusion, acute administration of ethanol enhances the extracellular levels of 5-HT in the vHIP of P rats, but not NP rats, in a dose-related manner. However, unlike what has been previously reported in Wistar rats, P rats do not develop rapid tolerance to ethanol-stimulated increases in extracellular levels of 5-HT in the vHIP. Additional studies will be necessary to see if P rats can develop tolerance to the ethanol-stimulated increase in 5-HT levels in the vHIP after chronic ethanol exposure.

Acknowledgments

We gratefully acknowledge the technical assistance of Thomas Zinski, Allison R. Jones, Tiffany E. Hill, and Carol Kulesavage. This study was supported in part by NIAAA Grant Nos. AA07611 and AA10721.

References

Alexi T, Azmitia EC. Ethanol stimulates [3H]5-HT high-affinity uptake by rat forebrain synaptosomes: role of 5-HT receptors and voltage channel blockers. Brain Res 1991;544:243–7.

Azmitia EC, Segal M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 1978;179:641–67.

Bare DJ, McKinzie JH, McBride WJ. Development of rapid tolerance to ethanol-stimulated serotonin release in the ventral hippocampus. Alcohol: Clin Exp Res 1998;22:1272–6.

Chu N-S. Responses of midbrain raphe neurons to ethanol. Brain Res 1984;311:348–52.

Crabbe JC, Rigter H, Uijlen J, Strijbos C. Rapid development of tolerance to the hypothermic effect of ethanol in mice. J Pharmacol Exp Ther 1979;208:128–33.

Frankel D, Khanna JM, Kalant H, LeBlanc AE. Effect of *p*-chlorophenylalanine on the loss and maintenance of tolerance to ethanol. Psychopharmacology (Berlin) 1978a;56:139–43.

Frankel D, Khanna JM, Kalant H, LeBlanc AE. Effect of *p*-chlorophenylalanine on the acquisition of tolerance to the hypothermic effects of ethanol. Psychopharmacology (Berlin) 1978b;57:239–42.

Gatto GJ, Murphy JM, Waller MB, McBride WJ, Lumeng L, Li T-K. Persistence of tolerance to a single dose of ethanol in the selectively-bred alcohol-preferring P rat. Pharmacol, Biochem Behav 1987;28:105–10.

Justice JB. Quantitative microdialysis of neurotransmitters. J Neurosci Methods 1993;48:263–76.

Kalant H. Problems in the search for mechanisms of tolerance. Alcohol Alcohol, Suppl 1993;2:1–8.

Khanna JM, Kalant H, Shah G, Weiner J. Rapid tolerance as an index of chronic tolerance. Pharmacol, Biochem Behav 1991;38:427–32.

Khanna JM, Chau A, Shah G. Characterization of the phenomenon of rapid tolerance to ethanol. Alcohol 1996;13:621–8.

Khatib SA, Murphy JM, McBride WJ. Biochemical evidence for activation of specific monoamine pathways by ethanol. Alcohol 1988;5:295–9.

Kreiss DS, Lucki I. Differential regulation of serotonin (5-HT) release in the striatum and hippocampus by 5-HT_{1A} autoreceptors of the dorsal and median raphe nuclei. J Pharmacol Exp Ther 1994;269:1268–79.

Le A-D, Khanna JM, Kalant H, LeBlanc AE. Effect of L-tryptophan on the acquisition of tolerance to ethanol-induced motor impairment and hypothermia. Psychopharmacology (Berlin) 1979;61:125–9.

Le A-D, Khanna JM, Kalant H, LeBlanc AE. Effect of 5,7-dihydroxytryptamine on the development of tolerance to ethanol. Psychopharmacology (Berlin) 1980;67:143–6.

Le A-D, Khanna JM, Kalant H, LeBlanc AE. The effect of lesions in the dorsal, median and magnus raphe nuclei on the development of tolerance to ethanol. J Pharmacol Exp Ther 1981;218:525–9.

Lumeng L, Hawkins DT, Li T-K. New strains of rats with alcohol preference and nonpreference. In: Thurman RG, Williamson JR, Drott HR, Chance B, editors. Alcohol and aldehyde metabolizing systems, vol. 3. New York: Academic Press, 1977. pp. 537–44.

Lumeng L, Waller MB, McBride WJ, Li T-K. Different sensitivities to ethanol in alcohol-preferring and -nonpreferring rats. Pharmacol, Biochem Behav 1982;16:125–30.

McBride WJ, Murphy JM, Gatto GJ, Levy AD, Yoshimoto K, Lumeng L, Li T-K. CNS mechanisms of alcohol self-administration. Alcohol Alcohol, Suppl 1993;2:463–7.

Morzorati SL, Johnson TB. Serotonergic neuronal activity in the dorsal, raphe nucleus of selectivity bred alcohol-preferring and alcohol-nonpreferring rats and unselected Wistar rats. Alcohol: Clin Exp Res 1999;23:1362–7.

Murphy JM, McBride WJ, Lumeng L, Li T-K. Regional brain levels of monoamines in alcohol-preferring and -non preferring lines of rats. Pharmacol, Biochem Behav 1982;16:145–9.

Murphy JM, McBride WJ, Lumeng L, Li T-K. Contents of monoamines in forebrain regions of alcohol-preferring (P) and -nonpreferring (NP) lines of rats. Pharmacol, Biochem Behav 1987;26:389–92.

Murphy JM, McBride WJ, Gato GJ, Lumeng L, Li T-K. Effects of acute ethanol administration on monoamine and metabolite content in forebrain regions of ethanol-tolerant and -nontolerant alcohol-preferring (P) rats. Pharmacol, Biochem Behav 1988;29:169–74.

Parsons LH, Justice JB. Extracellular concentration and in vivo recovery of dopamine in the nucleus accumbens using microdialysis. J Neurochem 1992;58:212–8.

Parsons LH, Justice JB. Quantitative approaches to in vivo brain microdialysis. Crit Rev Neurobiol 1994;8:189–220.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. San Diego: Academic Press, 1998.

Portas CM, Devoto P, Gessa GL. Effect of ethanol on extracellular 5-hydroxytryptamine output in rat frontal cortex. Eur J Pharmacol 1994; 270:123–5.

Smith AD, Weiss F. Ethanol exposure differentially alters central monoamine neurotransmission in alcohol-preferring versus -nonpreferring rats. J Pharmacol Exp Ther 1999;288:1223–8.

Steinbusch HW, Nieuwenhuys R. The raphe nuclei of the rat brainstem: a cytoarchitectonic and immunohistochemical study. In: Emson PC, editor. Chemical neuroanatomy. New York: Raven Press, 1983. pp. 131–207.

Stewart RB, Kurtz DL, Zweifel M, Li T-K, Froehlich JC. Differences in the hypothermic response to ethanol in rats selectively bred for oral ethanol preference and nonpreference. Psychopharmacology (Berlin) 1992; 106:169–74.

Thielen RJ, Morzorati SL, McBride WJ. Effects of ethanol on the dorsal raphe nucleus and its projections to the caudate putamen. Alcohol 2001;23:131–9.

Waller MB, McBride WJ, Lumeng L, Li T-K. Initial sensitivity and acute tolerance to ethanol in the P and NP lines of rats. Pharmacol, Biochem Behav 1983;19:683–6.

Weiss F, Lorang MT, Bloom FE, Koob GF. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 1993;267: 250–8.

Yoshimoto K, McBride WJ, Lumeng L, Li T-K. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 1992a;9:17–22.

Yoshimoto K, McBride WJ, Lumeng L, Li T-K. Ethanol enhances the release of dopamine and serotonin in the nucleus accumbens of HAD and LAD lines of rats. Alcohol: Clin Exp Res 1992b;16:781–5.

Zhou FC, Bledsoe S, Lumeng L, Li T-K. Immunostained serotonergic fibers are decreased in selected brain regions of alcohol-preferring rats. Alcohol 1991;8:425–31.

Zhou FC, Bledsoe S, Lumeng L, Li T-K. Reduced serotonergic immunoreactive fibers in the forebrain of alcohol-preferring rats. Alcohol: Clin Exp Res 1994a;18:571–9.

Zhou FC, Pu CF, Murphy JM, Lumeng L, Li T-K. Serotonergic neurons in the alcohol preferring rats. Alcohol 1994b;11:397–403.