ELSEVIER

Biochemical Pharmacology 62 (2001) 1025–1036

Biochemical Pharmacology

# Induction of CYP2B1/2 and nicotine metabolism by ethanol in rat liver but not rat brain

Kerri A. Schoedel[a], Edward M. Sellers[a,b,c], Rachel F. Tyndale[a,c,*]

[a]*Department of Pharmacology, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada M5S 1A8*
[b]*Department of Psychiatry, University of Toronto, Clark Institute, 250 College St., Toronto, Ontario, Canada M5S 2S1*
[c]*Centre for Addiction and Mental Health, University of Toronto, 33 Russell St., Toronto, Ontario, Canada M5S 2S1*

Received 11 July 2000; accepted 15 February 2001

**Abstract**

A higher proportion of alcoholics than non-alcoholics smoke (>80 vs 30%). In animals, chronic administration of alcohol induces tolerance to some effects of nicotine. To investigate if chronic ethanol (EtOH) induces alterations in CYP2B1/2 and nicotine C-oxidation activity, male rats (N = 4–6/group) were treated once daily with saline or EtOH (0.3, 1.0, and 3.0 g/kg, p.o./by gavage) for 7 days. A quantitative immunoblotting assay was developed to detect CYP2B1/2 in the brain, where constitutive expression is low, and in the liver. Using this method, it was determined that EtOH did not alter CYP2B1/2 protein expression significantly in six brain regions (olfactory bulbs, olfactory tubercles, frontal cortex, hippocampus, cerebellum, and brainstem). However, a dose-dependent induction of CYP2B1/2 protein expression was detected in the liver. Significant induction of 2-, 3-, and 2.7-fold were observed for the 0.3, 1.0, and 3.0 g/kg doses, respectively. Increases were also observed in CYP2B1 mRNA, which was induced by 14, 38, and 43% at the same doses. Liver microsomal nicotine C-oxidation also was increased (1.3 to 4.5-fold). CYP2B selective inactivators demonstrated that approximately 70% of nicotine C-oxidation was mediated by CYP2B1/2 in both EtOH-induced and uninduced hepatic microsomes. In summary, chronic, behaviorally relevant doses of EtOH induce CYP2B1/2 protein, mRNA, and nicotine C-oxidation activity in rat liver but not in rat brain, and these increases could contribute to cross-tolerance and co-abuse of ethanol and nicotine. 

*Keywords:* Cytochromes P450; CYP2B; Nicotine metabolism; Ethanol; Cross-tolerance; Rat liver

## 1. Introduction

CYPs are a family of heme-containing drug-metabolizing enzymes that are involved in the biotransformation of xenobiotics, environmental contaminants, dietary components, and procarcinogens [1–3]. Rat *CYP2B1/2*, mouse *Cyp2b9/10*, and human *CYP2B6* share approximately 80% nucleotide sequence identity [4]. CYP2B enzymes metabolize a diverse group of compounds including pesticides [5], chemotherapeutics such as cyclophosphamide [6], tobacco-specific nitrosamines (NNK, NMA, NDMA) [7,8], tricyclic antidepressants [4], drugs of abuse such as cocaine [9], and nicotine [10]. In humans, nicotine is metabolized primarily by hepatic CYP2A6 [11], due to low and variable expression of CYP2B6, which has higher nicotine-metabolizing activity [12]. In rats, nicotine is metabolized primarily by CYP2B1/2, which also has a low level of constitutive expression; rat CYP2A enzymes do not metabolize nicotine [13].

Hepatic CYP2B enzymes are inducible by a variety of structurally diverse compounds such as barbiturates [14], pesticides [15], acetone, isosafrole, and pregnenolone-16$\alpha$-carbonitrile [16]. Induction of CYP2B enzymes has been correlated with tumor promotion and increased hepatocarcinogenesis [17,18], perhaps due to their role in the activation of a number of procarcinogens such as aminoanthracene, benzo[*a*]pyrene [16], and tobacco-specific nitrosamines such as NDMA, NNK, and NMA [7,8].

CYP2B enzymes are also expressed in the brain [19]. CYP2B1/2 enzymes are functional in this organ [20], and are expressed in a region- and cell-specific manner at levels approximately 100-fold lower than in the liver [21]. The

* Corresponding author. Tel.: +1-416-978-6374; fax: +1-416-978-6395.

*E-mail address:* r.tyndale@utoronto.ca (R.F. Tyndale).

*Abbreviations:* CYP, cytochrome P450; C8 xanthate, potassium octylxanthate; NCO, nicotine C-oxidation; NDMA, *N*-nitrosodimethylamine; NMA, *N*-nitroso-*N*-methylaniline; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; and SSC, saline–sodium citrate buffer.

0006-2952/01/$ – see front matter 
PII: S0006-2952(01)00744-4

expression of CYP2B1/2 can be modulated by centrally acting agents, and may alter the local metabolism of these drugs at their site of action [22]. For example, chronic nicotine can increase CYP2B1/2 protein and activity in several brain regions but not in the liver [23,24]. This could lead to increased nicotine clearance in close proximity to central nicotinic receptors.

A much higher proportion of alcoholics smoke (80–95%) relative to non-alcoholics (25–30%), and 70% of those are heavy smokers [for a review, see Ref. 25]. Alcoholics, as well as social drinkers, consume more cigarettes per day than non-alcohol-consuming smokers [26–28]. Recently, nicotine and alcohol dependence were shown to have substantial common genetic susceptibility, suggesting common underlying causes for the two [29]. Alcoholics report marked tolerance to nicotine and more severe symptoms of nicotine withdrawal when compared with non-alcohol-dependent smokers [30]. Cross-tolerance between ethanol and nicotine has also been observed in animal models [31]. Because smokers titrate their smoking behavior to maintain plasma and central nicotine levels [32], tolerance can lead to increased consumption [25].

Mechanisms by which cross-tolerance occur include changes in target receptors or altered drug metabolism. While acute ethanol appears to stabilize nicotinic receptors in the desensitized state, changes in [$^{3}$H] nicotine and [$^{125}$I] $\alpha$-bungarotoxin binding to central nicotinic receptors appear to be modest or non-existent, even after prolonged ethanol administration [31,33,34]. CYP2B1/2 protein and/or mRNA increase after prolonged dietary consumption of high-dose ethanol [35]; however, it is not known whether this occurs at behaviorally relevant doses, whether this result is dose-dependent, or if the increase in CYP2B1/2 protein specifically translates into increased metabolism of nicotine.

The aim of this study was to determine whether short-term, behaviorally relevant doses of ethanol increase CYP2B1/2 protein and mRNA in rat brain and/or liver. A second aim was to determine if the ethanol-induced increases in CYP2B1/2 expression resulted in changes in nicotine metabolism; peripheral or central increases in nicotine metabolism could contribute to the increased smoking observed in alcoholics and social drinkers.

## 2. Materials and methods

### *2.1. Materials*

Protease inhibitor tablets and Chemiluminescence Blotting Substrate were purchased from Roche Diagnostics. Recombinant baculovirus-expressed CYP2B1 Supersomes and phenobarbital-induced rat liver microsomes were purchased from the Gentest Corp. Protran™ nitrocellulose membranes were purchased from Schleicher & Schuell Inc. Rat tissue adsorbed biotinylated anti-mouse IgG secondary antibody was purchased from Vector Laboratories Inc., and Neutravidin™-conjugated horseradish peroxidase was purchased from the Pierce Chemical Co. The protein assay kit, pre-stained molecular markers, and Zeta-Probe nylon membrane were purchased from Bio-Rad Laboratories. A Strataprep Total RNA Mini-prep kit was purchased from Stratagene. Yeast tRNA was purchased from GibcoBRL. C8 xanthate was custom synthesized by Toronto Research Chemicals. 5-Methylnicotine was provided by Peyton Jacob III (University of California). Nicotine bitartrate and 8-methoxypsoralen were purchased from Sigma-Aldrich Canada Ltd.

### *2.2. Animals*

Saline, and ethanol (in saline) at doses of 0.3, 1.0, and 3.0 g/kg body weight (N = 4–6 per group), was administered by gavage to adult male Wistar rats (250–300 g; Charles River) once daily for 7 days. Animals were decapitated 4 hr following administration of the last drug dose. Livers were removed, frozen immediately in liquid nitrogen, and stored at −80° until processed for RNA, protein, and activity studies. Brains were dissected on ice into regions (olfactory bulbs, olfactory tubercles, frontal cortex, hippocampus, cerebellum, and brainstem, according to Paxinos and Watson's stereotaxic atlas of the brain [36]), immediately frozen in liquid nitrogen, and stored at −80°. These brain regions were studied because they express nicotinic receptors, and are involved in mediating the central effects of ethanol and nicotine [37,38]. All experimental procedures used in this study were carried out in accordance with guidelines for the care and use of laboratory animals and approved by the Animal Care Committee of the University of Toronto.

### *2.3. Membrane preparation*

For immunoblotting, whole membrane preparations were used because CYPs in the brain, including CYP2B1/2 are located in both the microsomal and mitochondrial fractions [23,39]. Membranes of liver tissue and brain regions were prepared as described previously [23], aliquoted into small volumes, and stored at −80°.

For nicotine metabolism studies, hepatic microsomal preparations were prepared according to established techniques [40]. Protein concentrations were determined according to the instructions of the manufacturer. The cytosolic fractions were pooled and used as a source of aldehyde oxidase [11].

### *2.4. Immunoblotting*

To determine the linear range of detection for the assay, untreated brainstem and liver membranes were diluted serially and used to construct standard curves. Membrane proteins from brain regions and livers (40 $\mu$g) were separated by SDS–PAGE (4% stacking and 8% separating gels), and transferred overnight onto nitrocellulose membranes.

Insect-cell expressed CYP2B1 and phenobarbital-induced rat liver microsomes were used as standards. Membranes were preincubated for 1 hr in a blocking solution containing 3% skim milk powder (w/v), 1% BSA (w/v), and 2% normal rat serum (v/v) in TBST [50 mM Tris (pH 7.4), 150 mM NaCl, 0.1% (v/v) Triton X-100]. Membranes were probed with a monoclonal antibody to rat CYP2B1 (1:3000 dilution), which has been characterized previously [41] (provided by Harry V. Gelboin, NCI, NIH). Membranes were incubated with a secondary (rat tissue adsorbed) biotinylated anti-mouse antibody (1:2000 dilution), followed by incubation with a tertiary Neutravidin™-conjugated horseradish peroxidase (1:30,000 dilution). All antibodies were suspended in a solution of 1% BSA (w/v) in TBST. Membranes were washed in TBST following each antibody incubation step. Control blots were incubated without primary antibody. Protein bands were detected by enhanced chemiluminescence. Membranes were exposed to Kodak X-OMAT-AR film for 0.5 to 2 min. Immunoblots were analyzed using an imaging system (Imaging Research Inc.).

### 2.5. RNA analysis

RNA was isolated using guanidinium thiocyanate [42] as described by the supplier (Stratagene). RNA quantity and quality were analyzed by the ratio of optical density (260/280 UV wavelength), and agarose gel electrophoresis. For slot blot analysis, a standard curve (0.03 to 0.25 $\mu$g) was generated using total RNA isolated from a phenobarbital-treated rat liver expressing high levels of CYP2B mRNA [43]. Signals detected from all samples were within the linear range of the system. Initial experiments indicated that addition of yeast tRNA to total RNA reduced background and improved the linearity of the system. Therefore, yeast tRNA was added to all samples such that the final amount of RNA in each well was equal to 10 $\mu$g (5.0 to 7.5 $\mu$g of tRNA was added to 2.5 to 5.0 $\mu$g of total RNA). RNA pooled from four animals (by amount of total RNA) was applied directly to nylon membranes under vacuum and denaturing conditions using a Bio-Dot microfiltration apparatus according to the instructions of the manufacturer. Membranes were preincubated (>60 min) at 43° in prehybridization buffer [50% formamide, 120 mM $NA_2HPO_4$ (pH 7.2), 7% SDS, and 250 mM NaCl], and hybridized ($1 \times 10^7$ cpm in 5 mL of pre-hybridization buffer) with a random-primed ([$^{32}$P]dCTP) full-length rat CYP2B1 cDNA generously (provided by Curtis J. Omiecinski, University of Seattle) for 16 hr at 43°. Blots were washed at room temperature for 15 min, each sequentially in 2× SSC with 0.1% SDS, 0.5× SSC with 0.1% SDS, and 0.1× SSC with 0.1% SDS, and then exposed to Kodak X-OMAT-AR film for 24−48 hr at −80°. Blots were then stripped of probe by washing twice for 5 min each at 95° in 0.1× SSC with 0.5% SDS. Re-hybridization proceeded as described above, using a randomly-labeled rat $\beta$-actin probe. All films were analyzed using an imaging system from Imaging Research Inc.

### 2.6. NCO activity and chemical inhibition assays

NCO activity was assayed according to the method of Messina *et al.* [11] for human liver microsomes with the following modifications. Incubation mixtures contained 1 mM NADPH and 20 $\mu$L of rat liver cytosol (a source of aldehyde oxidase) in Tris–HCl buffer (pH 7.4). Incubations were carried out at 37°. The reaction was stopped, 5-methylnicotine (50 $\mu$L of 2 $\mu$g/mL) was added as the internal standard, samples were extracted with 3 mL dichloromethane, and the organic phase was dried under nitrogen. Samples were reconstituted with 200 $\mu$L of 0.01 M HCl, and 50 $\mu$L of each sample was subjected to HPLC analysis with a UV detector (set at 260 nm). Separation of nicotine and metabolites was achieved using a Supercosil LC-8DB column (5 $\mu$m, 150 × 4.6 mm, Supelco) and a mobile phase consisting of acetonitrile:potassium phosphate buffer [10:90 (v/v), pH 4.6] containing 1 mM octanesulfonic acid and 0.5% (v/v) triethylamine. The separation was performed with isocratic elution at a flow rate of 1 mL/min. The retention times for nicotine, cotinine, and 5-methylnicotine were 4.1, 5.1, and 7.4 min, respectively. CYP2B1 nicotine-to-cotinine kinetics were performed by incubating 0.55, 1, 1.7, 3, 5.5, 10, 1.7, 30, 55, and 100 $\mu$M (*S*)-nicotine with 5 pmol of expressed CYP2B1 for 20 min. Standard curves were created for cotinine (0.13 to 4.2 $\mu$M). Chemical inhibition studies were conducted in conjunction with nicotine assays. Samples of 1.0 mg of hepatic microsomal protein (pooled from four animals by protein concentration) or 5 pmol expressed CYP2B1 were preincubated with either 24 $\mu$M C8 xanthate or 29 $\mu$M 8-methoxypsoralen, or vehicle controls (HCl buffer or methanol, respectively) for 15 min at 30°. Incubations also contained 20 $\mu$L rat cytosol, 1 mM NADPH, and Tris–HCl buffer. Nicotine metabolism was then initiated with 25 $\mu$M (*S*)-nicotine, and proceeded as described above. Nicotine and inhibitor concentrations were chosen based on $K_m$ and $K_i$ values for the compounds (approximately $3.5 \times K_m$, and $10 \times K_i$ for nicotine and inhibitors, respectively [44,45]). Negative controls consisted of incubations without NADPH or with heat-denatured microsomal protein.

### 2.7. Statistics

Treatment groups were considered to be significantly different from control if $P < 0.05$, using unpaired Student's *t*-tests. Correlation analysis was performed using a Pearson correlation co-efficient (R value) followed by a Fisher z-test.

## 3. Results

### 3.1. CYP2B1 in rat brain

An immunoblotting assay was developed in order to detect CYP2B1/2 in brain tissue where constitutive expres-

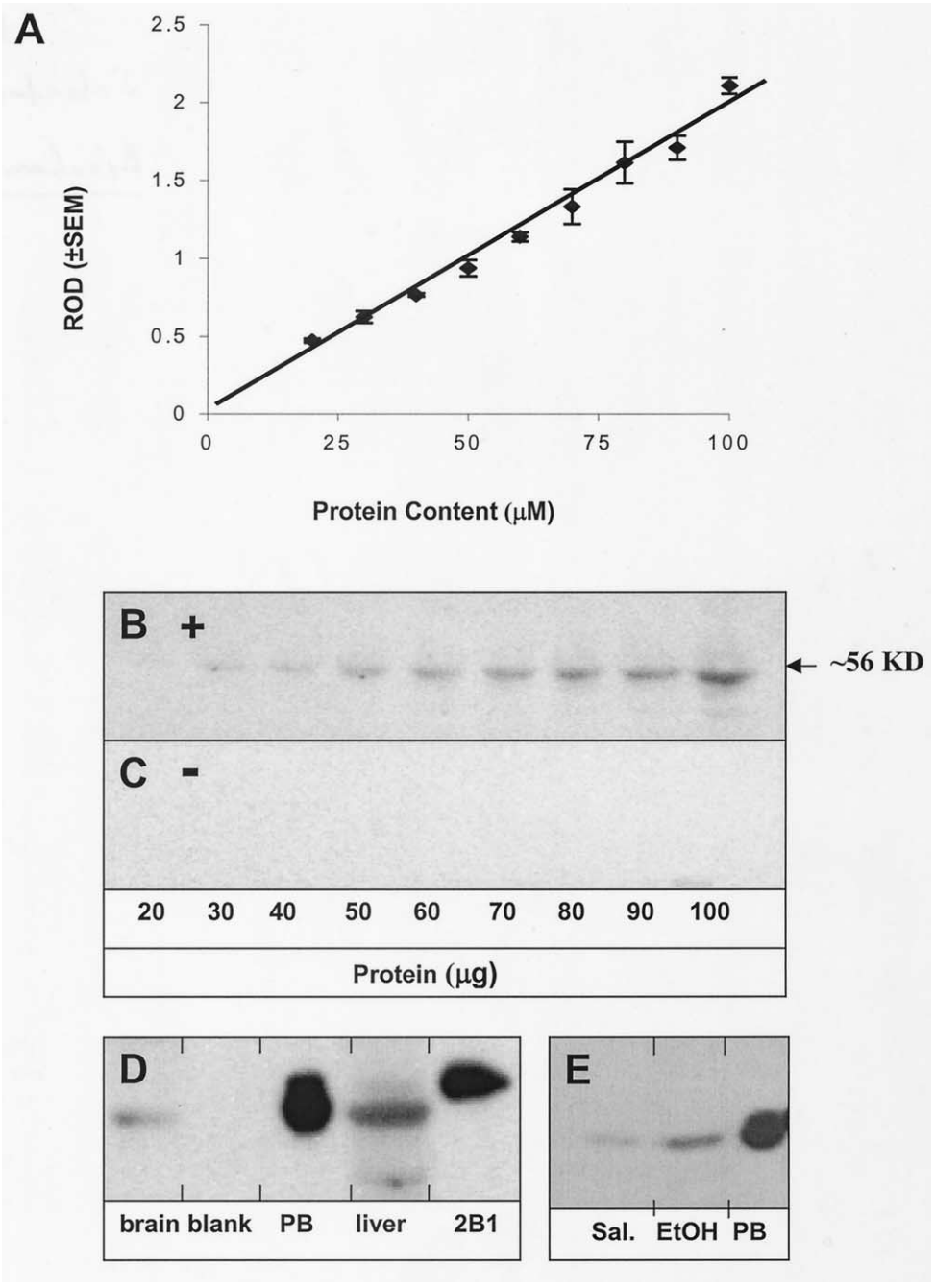


Fig. 1. Immunoblotting assay for CYP2B in rat brain. (A) A dilution curve of untreated rat brainstem CYP2B1/2 protein detected by western blotting (± SEM, 4 experiments). (B and C) Representative immunoblots incubated with (B) and without (C) primary antibody. (D) Immunodetectable CYP2B1 protein from brain co-migrated with a band from untreated and phenobarbital-treated (PB) rat liver microsomes. The migration of insect-cell expressed CYP2B1 (2B1) was slightly slower. The blank contained only loading buffer. (E) Relative induction of CYP2B1 by ethanol (2.5-fold), and PB (approximately 150-fold). ROD = relative optical density, as determined by image analysis.

sion is low. The CYP2B1 reactive signal detected in these tissues was linear with the amount of protein loaded up to 100 $\mu$g of protein (Fig. 1, A and B), and selectivity was confirmed by incubating immunoblots without primary antibody (Fig. 1C). A CYP2B1 reactive signal could not be detected below 20 $\mu$g of brain membranes loaded. The immunoreactive band from brain tissues co-migrated with a band from untreated and phenobarbital-treated rat liver microsomes, while insect-cell expressed CYP2B1 migrated slightly more slowly (Fig. 1D). A second immunoreactive band of lower mobility was detected in phenobarbital-treated liver microsomes, and is probably CYP2B2 which is slightly larger than CYP2B1, with lower expression (approximately 10-fold lower, undetectable in brain tissue) [21,24]. Ethanol at the highest dose administered (3 g/kg) did not alter CYP2B1 levels significantly in six brain re-

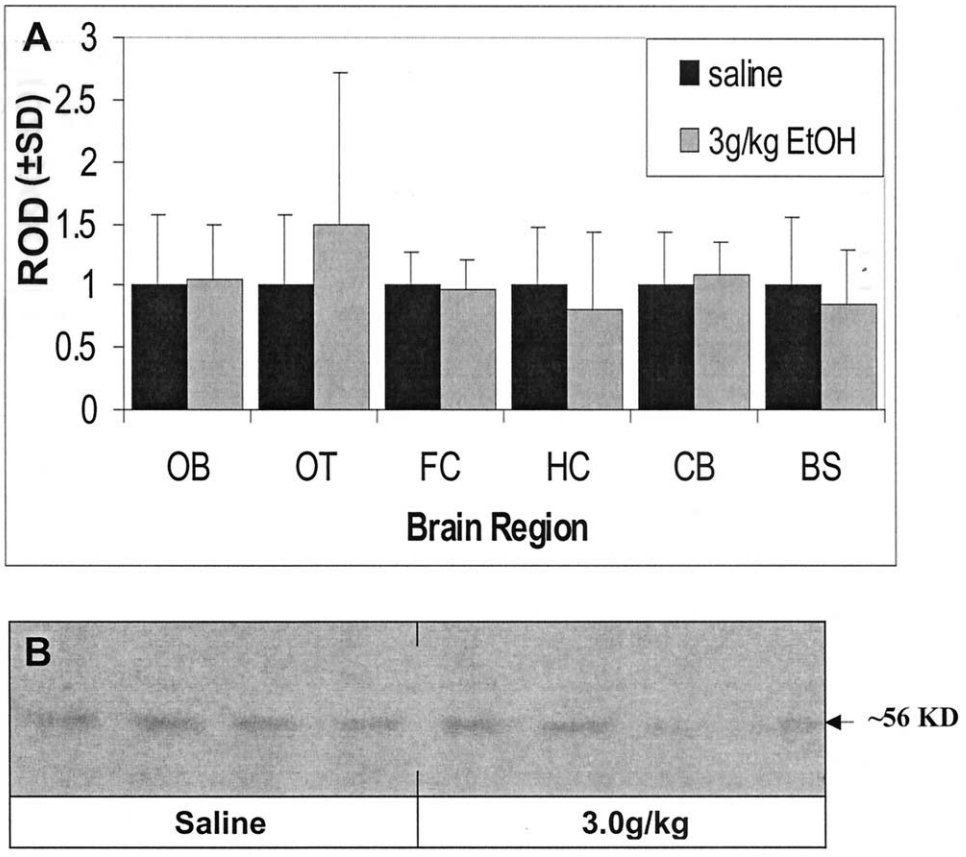


Fig. 2. CYP2B1/2 expression in six brain regions following chronic ethanol treatment. (A) Means (± SD) of six animals/group; saline means from each brain region were adjusted to equal 1 ROD unit, and standard deviations were adjusted by the same proportion. (B) Representative immunoblot from rat hippocampus, showing data from four saline and four ethanol-treated rats (3 g/kg), indicating the lack of induction by ethanol. Abbreviations: OB, olfactory bulbs; OT, olfactory tubercles; FC, frontal cortex; HC, hippocampus; CB, cerebellum; BS, brainstem.

gions (frontal cortex, olfactory bulbs, olfactory tubercles, hippocampus, cerebellum, and brainstem), when compared with saline controls (Fig. 2, A and B). A slight increase was observed for the olfactory tubercles, but this effect did not reach significance, most likely due to high interindividual variation (Fig. 2A).

### *3.2. Dose-dependent induction of CYP2B1 protein in rat liver by ethanol*

A serial dilution also was performed with untreated rat liver membranes (data not shown). Ethanol dose-dependently induced CYP2B1 protein in rat liver whole membranes (Fig. 3). CYP2B1 protein was increased by 2.0-fold ($P < 0.05$), 3.0-fold ($P < 0.001$), and 2.7-fold ($P < 0.001$) compared with saline controls for 0.3, 1.0, and 3.0 g/kg doses, respectively. The magnitudes of induction were similar to those observed for the ethanol-inducible CYP2E1 (1.6-, 1.9-, and 2.4-fold for the three doses, respectively) in the same animals.[1] By comparison, liver microsomes from phenobarbital-treated rats (commercial preparation, see Materials) showed approximately a 150-fold induction in CYP2B1 protein (Fig. 1E); although the assay was not optimized for this level of expression, this value is in agreement with reported values [43].

### *3.3. Dose-dependent induction of CYP2B1 mRNA in rat liver by ethanol*

Linear detection of CYP2B1 mRNA signal measured by slot blotting was established using serial dilution of phenobarbital-treated rat liver containing high levels of CYP2B mRNA [43]; all subsequent measurements were within the linear range of the assay (Fig. 4A). No detectable signal was observed with tRNA alone. CYP2B1 mRNA was induced significantly by chronic ethanol at all three doses (Fig. 4, B and D). Significant increases of 1.14-, 1.38-, and 1.43-fold (relative to saline) were observed for 0.3 ($P < 0.05$), 1.0 ($P < 0.001$), and 3.0 ($P < 0.001$) g/kg of ethanol, respectively (Fig. 4, B and D). Phenobarbital-treated rat liver RNA (used to construct the serial dilution curve and to serve as a positive control) showed a 25-fold induction of CYP2B1 mRNA (Fig. 4A). Blots were stripped and re-probed for $\beta$-actin mRNA (Fig. 4C), and no significant differences in

[1] Howard L and Tyndale R, unpublished results.

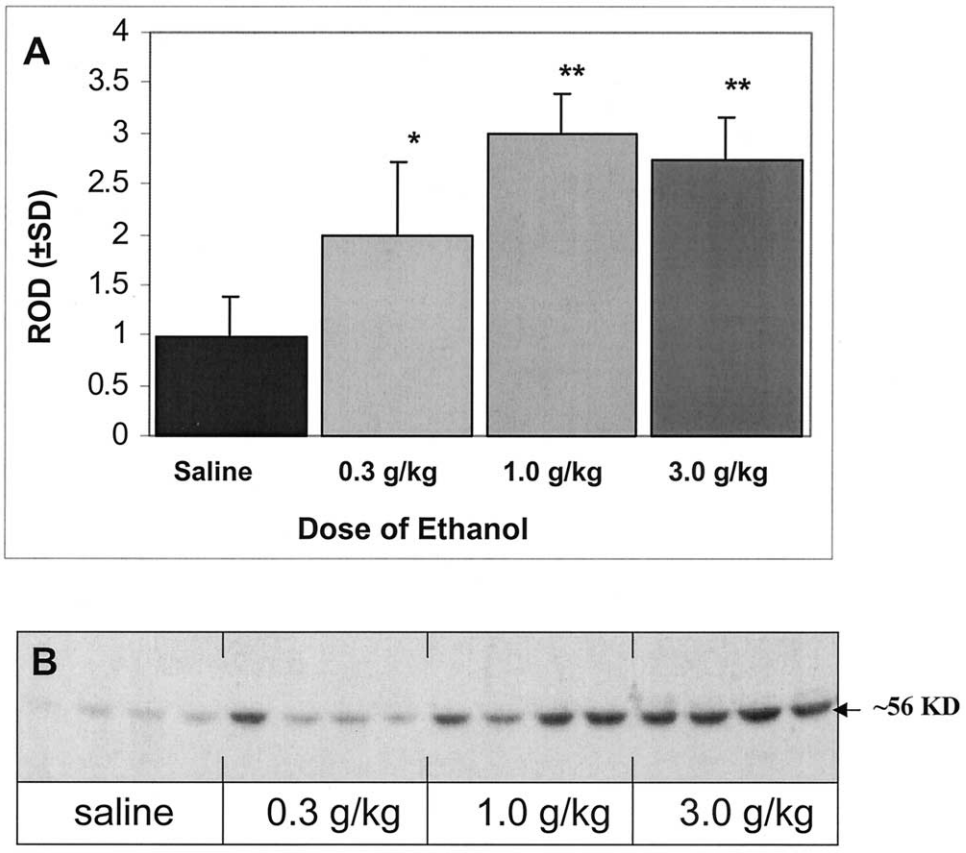


Fig. 3. Dose-dependent induction of CYP2B1 protein by ethanol in rat liver. (A) Dose–response for saline and three doses of ethanol; means (± SD) of four animals/group, and (B) representative immunoblot. The saline group was adjusted to equal 1 ROD unit; other groups (± SD) were adjusted by the same proportion. Significant differences from saline are indicated by: (*) $P < 0.05$, and (**) $P < 0.001$.

loading were detected between the saline and the ethanol treatment groups (ANOVA, $P = 0.34$). CYP2B mRNA levels correlated significantly with protein expression across the four treatment groups ($R = 0.96$, $P = 0.05$).

### *3.4. Induction of nicotine metabolism by ethanol in rat liver microsomes*

Microsomes from the same livers used in the preceding experiments were analyzed for NCO to the major metabolite cotinine. Initial experiments determined protein concentrations and incubation times that resulted in linear production of cotinine with less than 15% nicotine disappearance. Microsomes incubated without NADPH or heat-denatured microsomes produced no detectable cotinine. Expressed CYP2B1 had a $K_m$ of 7 $\mu$M and a $V_{max}$ of 0.074 $\mu$mol cotinine/pmol CYP2B1/min (Fig. 5).

Ethanol dose-dependently induced NCO activity in rat liver microsomes (Fig. 6A). At 25 $\mu$M nicotine (approximately 3.5 times the $K_m$), NCO was increased by 2.4-fold ($P < 0.005$), 1.3-fold ($P < 0.05$), and 4.5-fold ($P < 0.05$) compared with saline in the 0.3, 1.0, and 3.0 g/kg ethanol-treated microsomes, respectively (Fig. 6A). Nicotine metabolism was only weakly correlated to CYP2B1/2 protein for saline and the three ethanol doses ($R = 0.43$, $P = 0.65$). This was due primarily to the relatively low induction of nicotine metabolism (1.3-fold) for the 1.0 g/kg dose (Fig. 6A); the cause of this is unknown. The correlation between nicotine metabolism and CYP2B1 protein increased considerably when this dose was not included in the analysis ($R = 0.98$, $P < 0.05$). A similar relationship was observed for CYP2B1/2 mRNA and NCO ($R = 0.59$, $P = 0.5$, and $R = 0.99$, $P < 0.005$ with and without the 1.0 g/kg dose, respectively).

### *3.5. Inhibition of nicotine metabolism by CYP2B1-selective inhibitors*

At 25 $\mu$M nicotine expressed CYP2B1 metabolized nicotine to cotinine with a velocity of 16.2 ± 8.1 nmol cotinine formed per pmol CYP2B1 per min. At 1 mg protein, control rat liver microsomes metabolized nicotine to cotinine with a velocity of 19.5 ± 8.7 nmol cotinine formed per mg protein per min. Using the same incubation conditions with 5 pmol of CYP2B1 (resulting in approximately 5 times the production of cotinine), expressed CYP2B1-mediated NCO was abolished completely by the CYP2B1/2 selective mechanism-based inactivator C8 xanthate [44] and significantly

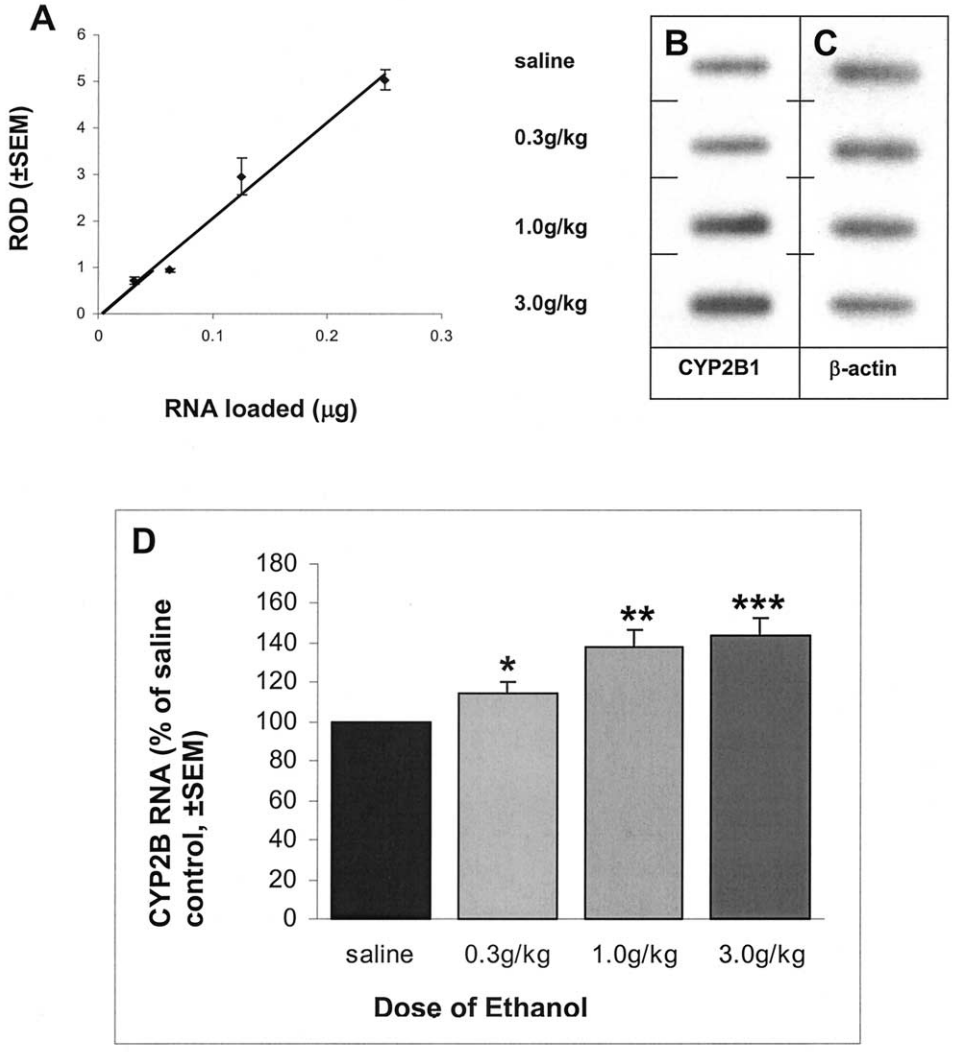


Fig. 4. Induction of CYP2B1 mRNA by ethanol in rat liver. (A) Dilution curve of phenobarbital-induced rat liver demonstrating linear detection of CYP2B RNA (means ± SEM, of 3 experiments). (B) Representative image of CYP2B mRNA detected by slot blotting. (C) Corresponding image of B re-probed for $\beta$-actin mRNA demonstrating equal loading of total RNA. (D) Dose–response for saline and three doses of ethanol. Increases in total CYP2B1/2 mRNA are expressed as a percentage of the saline control (means ± SEM, of 9 experiments). Significant differences from saline are indicated by: (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$.

inhibited by 8-methoxypsoralen, a CYP2B and CYP2A-selective inhibitor [45] (Fig. 6B, $P < 0.05$). Inhibitor concentrations chosen were approximately 10 times the $K_i$ values ($K_i$ of 2.4 and 2.9 $\mu$M for C8 xanthate and 8-methoxysporalen, respectively [44,45]) to ensure complete inactivation of CYP2B1 in liver microsomes. C8 xanthate significantly inhibited ($P < 0.05$) NCO by 77, 67, 67, and 75% for saline, 0.3, 1.0, and 3.0 g/kg ethanol, respectively (mean 71.4 ± 5.1%), suggesting that this portion of NCO is mediated by CYP2B1 (Fig. 6A). The inhibition was more variable for 8-methoxypsoralen, with values of 58, 73, 67, and 75% (all $P < 0.05$) inhibition for saline and the three ethanol doses (mean of 68.4 ± 6.9%). The relatively constant proportion of NCO inhibited by CYP2B1-selective inactivators across treatment groups suggests that chronic ethanol induces a non-CYP2B component of NCO, which is also present in saline-treated controls.

## 4. Discussion

In this study, behaviorally relevant doses of chronic ethanol were chosen to model light social drinking to heavy binge drinking by alcoholics [46] over a relatively short time course of 7 days. Previous studies [35] examining the effects of ethanol on cytochromes P450 employed periods of 20–30 days using the Lieber–DeCarli liquid ethanol diet [47], which provides approximately 12–13 g/kg per day of ethanol [48]. In rats, the dose of 0.3 g/kg of ethanol represents approximately 1–2 drinks per day (12–25 g of ethanol; 0.2–0.4 g/kg for a 70 kg individual) equivalent to light social drinking in humans. The dose of 1.0 g/kg represents slightly more, approximately 3–6 drinks per day (40–80 g of ethanol; 0.6–1.1 g/kg). The dose of 3.0 g/kg is approximately equivalent to 12–14 drinks per day (150–190 g;

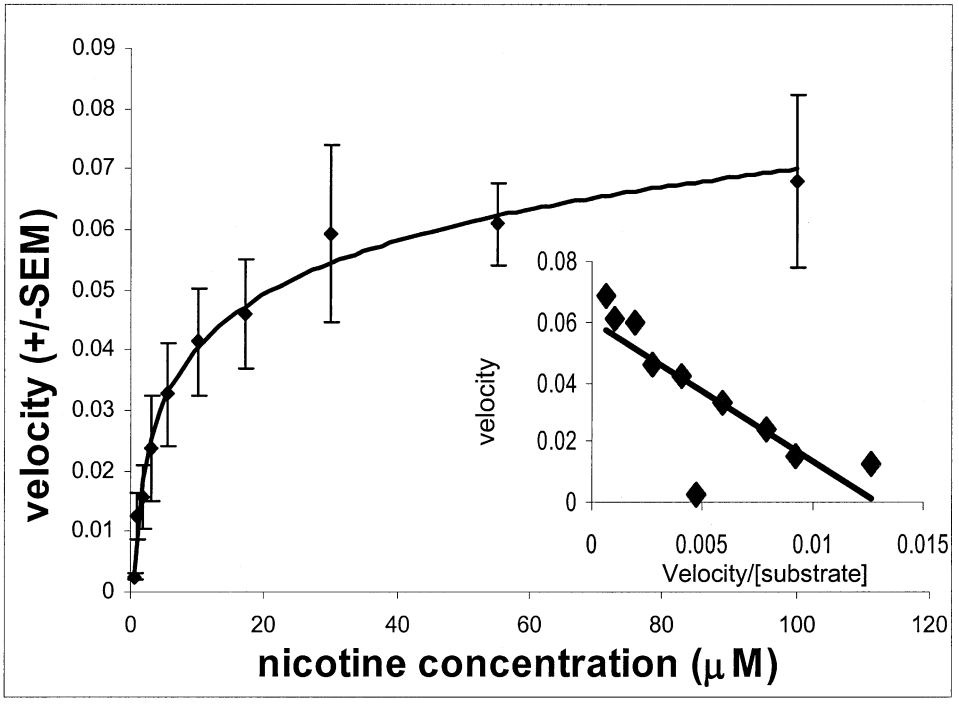


Fig. 5. Nicotine metabolism to the inactive metabolite cotinine by expressed CYP2B1. Expressed CYP2B1 metabolizes nicotine to cotinine with a $K_m$ of 7 $\mu$M and a $V_{max}$ of 0.074 $\mu$mol cotinine/pmol CYP2B1/min. An Eadie-Hofstee plot is shown in the inset. Velocity is expressed as $\mu$mol cotinine/pmol CYP2B1/min.

2.1–2.7 g/kg), which models the heavy drinking often seen with chronic alcoholics.

Chronic nicotine induces CYP2B1/2 in several brain regions, including olfactory bulbs, olfactory tubercles, frontal cortex, and brainstem [23]. We were therefore interested in determining if chronic ethanol also affects CYP2B1/2 in rat brain, possibly accounting for a component of the cross-tolerance observed between ethanol and nicotine [31]. This could occur by inducing changes in local nicotine metabolism at the site of central nicotinic receptors. Therefore, brain regions known to contain nicotinic receptors and/or are sites of ethanol-induced neuronal changes were selected [37,38]. No effect of chronic ethanol on the selected regions (olfactory bulbs/tubercles, frontal cortex, hippocampus, cerebellum, and brainstem) was observed at the doses and duration used in this study. The dose of 3 g/kg of ethanol was sufficient to produce observable central effects, including sedation and ataxia. Even at 0.3 g/kg, significant increases in hepatic CYP2B were observed. Therefore, it is unlikely that the lack of effect was due to insufficient dosing or hepatic first-pass effects (oral route of administration).

In another study, while chronic nicotine increased CYP2B1 in rat brain, it had no effect on rat liver CYP2B1/2 [23]. Conversely, we found a dose-dependent increase in CYP2B1/2 protein by ethanol in rat liver but not rat brain. Although peripheral or central alterations in nicotine-metabolizing CYP2B1/2 by nicotine or ethanol could contribute to nicotine tolerance and cross-tolerance between these two drugs, our data suggest that regulatory control of CYP2B1 by these drugs differs. There is evidence that CYP2B1 can be regulated differently in different tissues. For example, acetone and phenytoin, which induce CYP2B enzymes in the liver, decrease CYP2B1 in rat nasal mucosa, and primary astrocytes, respectively [49,50]. The reasons for differential regulation of CYP2B1 by ethanol in different tissues are unknown, but it is possible that there are tissue-specific regulatory factors present in the liver but not in the brain.

Previous work on ethanol induction of hepatic CYP2B1 mRNA has been conflicting. One study demonstrated an increase in both CYP2B1 mRNA and protein [35], while another study did not detect a change in mRNA [51]. Our data, demonstrating a dose-dependent increase in CYP2B1 mRNA in response to chronic ethanol, confirm that the induction is occurring, at least in part, at the level of mRNA regulation.

This is the first report of an increase in hepatic nicotine metabolism following chronic ethanol treatment specifically related to induction of CYP2B1/2. Adir *et al.* [52] reported a decrease in plasma nicotine in rats after chronic treatment with a high dose of ethanol (8 g/kg). Although this effect was attributed to an increase in volume of distribution, this does not explain the observed increase in plasma cotinine in parallel with the decrease in plasma nicotine. Another group observed a slight decrease in nicotine metabolism in liver microsomes from ethanol-treated rats [53]. In humans, one study showed only a slight effect of ethanol on urinary nicotine clearance, and no effect on overall plasma clear-

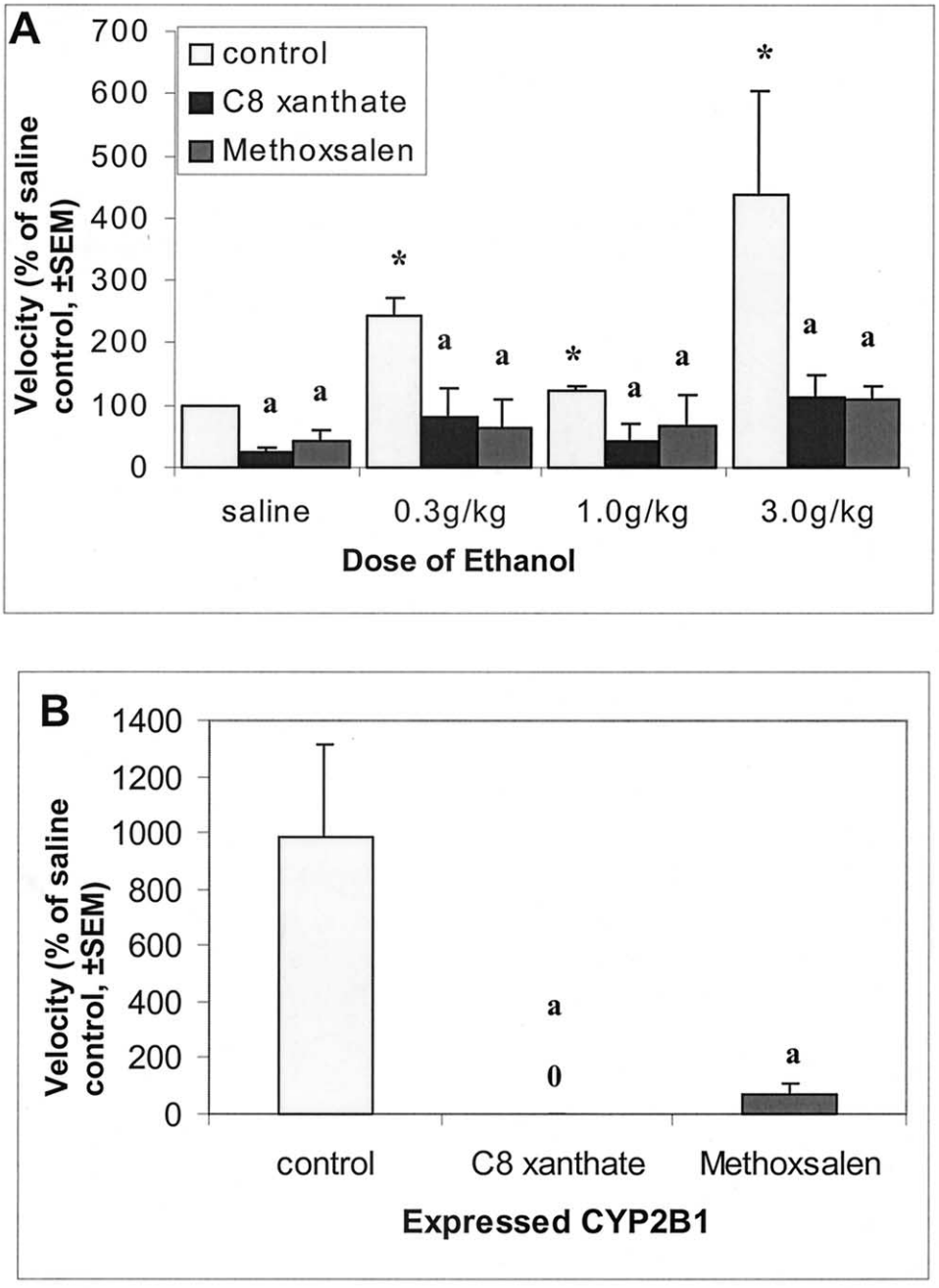


Fig. 6. Induction of NCO to cotinine by ethanol in rat liver. (A) NCO by liver microsomes from rats treated with saline and three doses of ethanol, expressed as a percentage of the saline control (± SEM). Values for percent inhibition by the CYP2B selective inactivator C8 xanthate were 77, 67, 67, and 75% for saline and the three doses of ethanol, respectively, and 58, 73, 67, and 75% for 8-methoxypsoralen. (B) NCO expressed CYP2B1 (5 pmol of CYP2B1), and inhibition by C8 xanthate and 8-methoxypsoralen. Key: (*) significant difference from saline, $P < 0.05$; and (a) significant inactivation by C8 xanthate and 8-methoxypsoralen (compared with control), $P < 0.05$.

ance; however, acute not chronic doses of ethanol were used [54]. Our study did show significant increases in nicotine metabolism at all three doses used. We used CYP2B-selective mechanism-based inactivators to show that approximately 70% of hepatic nicotine metabolism in rats was mediated by CYP2B1/2 in both uninduced and ethanol-induced livers. C8 xanthate is a very selective inactivator of CYP2B1 with a $K_i$ of 2.4 $\mu$M [44]. At higher concentrations, there is some inactivation of CYP2E1 ($K_i$ = 60 $\mu$M), an enzyme with no detectable NCO activity [13]. In our study, C8 xanthate (24 $\mu$M, 10 times the $K_i$) completely abolished NCO activity (25 $\mu$M nicotine, approximately 3.5 times the $K_m$) by expressed CYP2B1, at conditions that produced 5 times more cotinine than in the liver microsomal assays. While CYP2B2 is not inactivated by some compounds that inactivate CYP2B1 [55], it is not known

whether C8 xanthate also inactivates CYP2B2. However, CYP2B2 was not detected by immunoblotting, suggesting that 70% of NCO is mediated by CYP2B1 in rat liver. 8-Methoxypsoralen is slightly less selective, inactivating enzymes in the CYP2A as well as the CYP2B family [45]. This should not affect nicotine metabolism as CYP2A enzymes do not appreciably metabolize nicotine in rats [13]; however, this decreased selectivity may account for the increased variability in inhibition seen with this compound as compared with C8 xanthate. The finding that the fraction metabolized by CYP2B1 remained relatively constant in the induced and uninduced states suggests that the other enzymes involved in NCO are also being induced proportionately by ethanol. These could be CYP2C11, CYP2D1, and/or CYP1A2, which have low levels of NCO [13].

CYP2B1/2 metabolize a number of procarcinogens including the tobacco-specific nitrosamines NMA, NNK, and NDMA [7,8]. Induction of CYP2B enzymes has been correlated with increased tumor promotion and hepatocarcinogenesis [17,18]. Ethanol by itself is a weak carcinogen, but has a synergistic effect on tobacco-related cancers [56]. In fact, alcohol consumption stimulates CYP-mediated activation of tobacco-associated and dietary carcinogens in the liver, lungs, esophagus, and intestines [57]. Therefore, this could be another important consequence of ethanol induction of CYP2B enzymes.

CYP2B enzymes metabolize a number of clinically important drugs, including diazepam [4], buproprion [4], and chemotherapeutic pro-drugs such as cyclophosphamide [6]. Induction of hepatic CYP2B1/2 by ethanol could alter plasma drug levels. Therefore, in alcoholics this could lead to reductions in efficacy of drugs metabolized to inactive compounds. Conversely, pro-drugs could be metabolized to their active metabolite more quickly, leading to toxicity. This is particularly relevant for agents like cyclophosphamide, which have a very low therapeutic index [58]. CYP2B1/2 also metabolize cocaine to a toxic metabolite [9], suggesting additive or synergistic increases in liver damage when ethanol and cocaine are used concurrently. In fact, inhibition of CYP2B enzymes protects against cocaine-mediated hepatotoxicity in rats [9].

The mechanism(s) by which CYP2B enzymes are induced by ethanol is not known. Our observation that CYP2B1 mRNA is induced, as well as protein, suggests that the regulation is, in part, at the level of mRNA. CYP2B1 induction by a wide range of compounds occurs primarily at the transcriptional level [59]. This could occur by a number of mechanisms including increased transcription or mRNA stabilization. This mechanism has been described in detail for barbiturate induction [60–62]. It is not known whether this mechanism is involved in the ethanol induction of CYP2B1/2 observed here. The relatively low level of ethanol induction (3-fold) compared with the increases (10- to 100-fold) seen with barbiturates [43] suggests that the mechanisms may be different. In addition, we observed changes in mRNA that were quantitatively lower than the changes seen in protein. This could indicate a different mechanism such as increased translation or protein stabilization, or a combination of mechanisms at both the protein and mRNA level. The magnitudes of induction are similar to those observed for CYP2E1 in the same animals. Ethanol is thought to regulate CYP2E1 by protein stabilization [63]. In addition, the regulatory mechanism may not be a direct action of ethanol on CYP2B1/2 regulation. For example, chronic ethanol consumption alters hormonal secretion [64–66], and CYP2B expression is known to be modulated by a number of these different hormones including corticosteroids [60,67], insulin [68–71], and growth hormone [72–74]. Therefore, the specific mechanism(s) of induction of CYP2B enzymes by ethanol remains to be determined, but could include direct or indirect effects.

In summary, we showed that low, behaviorally relevant doses of chronically administered ethanol induced CYP2B1/2 protein, mRNA, and nicotine metabolism in a dose-dependent manner in the liver but not the brain of rats. Chronic ethanol could contribute to the cross-tolerance seen between ethanol and nicotine by increasing the clearance of nicotine. This may be an important factor leading to increased smoking by alcoholics. Due to procarcinogen activation by CYP2B enzymes, this could also be a contributing factor of the synergistic effect of alcohol on smoking-related cancers. In addition, differences in the metabolism of clinically important CYP2B substrates may be observed in alcoholics versus non-alcoholics.

## Acknowledgments

We would like to thank Wenjiang Zhang, Sharon Miksys, Ewa Hoffmann, Yushu Rao, and Nick Basso for technical assistance. This work was funded by the Medical Research Council of Canada (MT14173), and the Centre for Addiction and Mental Health. K.A. Schoedel would like to thank the Centre for Addiction and Mental Health, the Ontario Graduate Scholarship program, and the Natural Sciences and Engineering Council of Canada for continued support.

## References

[1] Gonzalez FJ, Gelboin HV. Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. Drug Metab Rev 1994;26:165–83.

[2] Wrighton SA, Stevens JC. The human hepatic cytochromes P450 involved in drug metabolism. Crit Rev Toxicol 1992;22:1–21.

[3] Kivisto KT, Kroemer HK, Eichelbaum M. The role of human cytochrome P450 enzymes in the metabolism of anticancer agents: implications for drug interactions. Br J Clin Pharmacol 1995;40:523–30.

[4] Lewis DFV, Lake BG, Dickins M, Eddershaw PJ, Tarbit MH, Goldfarb PS. Molecular modelling of CYP2B6, the human CYP2B isoform, by homology with the substrate-bound CYP102 crystal structure: evaluation of CYP2B6 substrate characteristics, the cytochrome

$b_5$ binding site and comparisons with CYP2B1 and CYP2B4. Xenobiotica 1999;29:361–93.
[5] Dehal SS, Kupfer D. Metabolism of the proestrogenic pesticide methoxychlor by hepatic P450 monooxygenases in rats and humans. Dual pathways involving novel ortho ring-hydroxylation by CYP2B. Drug Metab Dispos 1994;22:937–46.
[6] Chang TKH, Weber GF, Crespi CL, Waxman DJ. Differential activation of cyclophosphamide and ifosphamide by cytochromes P450 2B and 3A in human liver microsomes. Cancer Res 1993;53:5629–37.
[7] Stiborová M, Hansíková H, Frei E. Metabolism of carcinogenic *N*-nitroso-*N*-methylaniline by purified cytochromes P450 2B1 and 2B2. Cancer Lett 1996;110:11–7.
[8] Code EL, Crespi CL, Penman BW, Gonzalez FJ, Chang TK, Waxman DJ. Human cytochrome P4502B6: interindividual hepatic expression, substrate specificity, and role in procarcinogen activation. Drug Metab Dispos 1997;25:985–93.
[9] Poet TS, Brendel K, Halpert JR. Inactivation of cytochromes P450 2B protects against cocaine-mediated toxicity in rat liver slices. Toxicol Appl Pharmacol 1994;126:26–32.
[10] Yamazaki H, Inoue K, Hashimoto M, Shimada T. Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. Arch Toxicol 1999;73:65–70.
[11] Messina ES, Tyndale RF, Sellers EM. A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J Pharmacol Exp Ther 1997;282:1608–14.
[12] Flammang AM, Gelboin HV, Aoyama T, Gonzalez FJ, McCoy GD. Nicotine metabolism by cDNA-expressed human cytochromes P450. Biochem Arch 1992;8:1–8.
[13] Nakayama H, Okuda H, Nakashima T, Imaoka I, Funae Y. Nicotine metabolism by rat hepatic cytochromes P450s. Biochem Pharmacol 1993;45:2554–6.
[14] Jones CR, Guengerich FP, Rice JM, Lubert RA. Induction of various cytochromes CYP2B, CYP2C and CYP3A by phenobarbitone in non-human primates. Pharmacogenetics 1992;2:160–72.
[15] Nims RW, Lubert RA, Fox SD, Jones CR, Thomas PE, Reddy AB, Kocarek TA. Comparative pharmacodynamics of CYP2B induction by DDT, DDE, and DDD in male rat liver and cultured rat hepatocytes. J Toxicol Environ Health 1998;53:455–77.
[16] Soucek P, Gut I. Cytochromes P-450 in rats: structures, functions, properties, and relevant human forms. Xenobiotica 1992;22:83–103.
[17] Rice JM, Diwan BA, Hu H, Ward JM, Nims RW, Lubert RA. Enhancement of hepatocarcinogenesis and induction of specific cytochrome P450-dependent monooxygenase activities by the barbiturates allobarbital, apobarbital, pentobarbital, secobarbital and 5-phenyl- and 5-ethylbarbituric acids. Carcinogenesis 1994;15:395–402.
[18] Diwan BA, Henneman JR, Nims RW, Rice JM. Tumor promotion by an anti-convulsant agent phenytoin in mouse liver: correlation with CYP2B induction. Carcinogenesis 1993;14:2227–31.
[19] Warner M, Köhler C, Hansson T, Gustafsson JÅ. Regional distribution of cytochrome P-450 in the rat brain: spectral quantitation and contribution of P450b,e, and P450c,d. J Neurochem 1988;50:1057–65.
[20] Parmar D, Dhawan A, Seth PK. Evidence for O-dealkylation of 7-pentoxyresorufin by cytochrome P450 2B1/2B2 isoenzymes in brain. Mol Cell Biochem 1998;189:201–5.
[21] Schilter B, Omiecinski C. Regional distribution and expression modulation of cytochrome P450 and epoxide hydrolase mRNAs in the rat brain. Mol Pharmacol 1993;44:990–6.
[22] Strobel HW, Geng J, Kawashima H, Wang H. Cytochrome P450-dependent biotransformation of drugs, and other xenobiotic substrates in neural tissue. Drug Metab Rev 1997;29:1079–105.
[23] Miksys S, Hoffmann E, Tyndale RF. Regional and cellular induction of nicotine-metabolizing CYP2B1 in rat brain by chronic nicotine treaent. Biochem Pharmacol 2000;59:1501–11.
[24] Anandatheerthavarada HK, Williams JF, Wecker L. The chronic administration of nicotine induces cytochrome P450 in rat brain. J Neurochem 1993;60:1941–4.
[25] Gordis E. Alcohol alert: alcohol and tobacco. Rockville, MD: National Institute on Alcohol Abuse and Alcoholism, National Institute of Health, 1998.
[26] Ayers J, Ruff CF, Templer DI. Alcoholism, cigarette smoking, coffee drinking, and extraversion. J Stud Alcohol 1976;37:983–5.
[27] Henningfield JE, Chait LD, Griffiths RR. Effects of ethanol on cigarette smoking by volunteers without histories of alcoholism. Psychopharmacology 1984;82:1–5.
[28] Mello NK, Mendelson JH, Palmieri SL. Cigarette smoking by women: interactions with alcohol use. Psychopharmacology 1987;93:8–15.
[29] True WR, Xian H, Scherrer JF, Madden PA, Bucholz KK, Heath AC, Eisen SA, Lyons MJ, Goldberg J, Tsuang M. Common genetic vulnerability for nicotine and alcohol dependence in men. Arch Gen Psychiatry 1999;56:655–61.
[30] Marks JL, Hill EM, Pomerleau CS, Mudd SA, Blow FC. Nicotine dependence and withdrawal in alcoholic and nonalcoholic ever-smokers. J Subst Abuse Treat 1997;14:521–7.
[31] Collins AC, Burch JB, de Fiebre CM, Marks MJ. Tolerance to and cross tolerance between ethanol and nicotine. Pharmacol Biochem Behav 1988;29:365–73.
[32] McMorrow MJ, Foxx RM. Nicotine's role in smoking: an analysis of nicotine regulation. Psychol Bull 1983;93:302–27.
[33] Collins AC, Booker TK. Long-term ethanol treatment elicits changes in nicotinic receptor binding in only a few brain regions. Alcohol 1997;14:131–40.
[34] Gorbounova O, Svensson AL, Jonsson P, Mousavi M, Miao H, Hellstrom-Lindahl E, Nordberg A. Chonic ethanol treatment decreases [$^3$H] epibatidine and [$^3$H] nicotine binding and differentially regulates mRNA levels of nicotinic acetylcholine receptor subunits expressed in M10 and SH-SY5Y neuroblastoma cells. J Neurochem 1998;70:1134–42.
[35] Johansson I, Ekstrom G, Scholte B, Puzycki D, Jornvall H, Ingelman-Sundberg M. Ethanol-, fasting-, and acetone-inducible cytochrome P-450 in rat liver: regulation, and characteristics of enzymes belonging to the IIB, and IIE gene subfamilies. Biochemistry 1988;27:1925–34.
[36] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press Ltd., 1986.
[37] Sziraki I, Sershen H, Benuck M, Hashim A, Lajtha A. Receptor systems participating in nicotine-specific effects. Neurochem Int 1998;33:445–57.
[38] Fadda F, Rossetti ZL. Chronic ethanol consumption: from neuroadaptation to neurodegeneration. Prog Neurobiol 1998;56:385–431.
[39] Perrin R, Minn A, Ghersi-Egea JF, Grassiot MC, Siest G. Distribution of cytochrome P450 activities towards alkoxyresorufin derivatives in rat brain regions, subcellular fractions and isolated cerebral microvessels. Biochem Pharmacol 1990;40:2145–51.
[40] Tyndale RF, Inaba T, Kalow W. Evidence in humans for variant allozymes of the nondeficient sparteine/debrisoquine monooxygenase (P450IID 1) *in vitro*. Drug Metab Dispos 1989;17:334–40.
[41] Goldfrab I, Korzekwa K, Krausz KW, Gonzalez F, Gelboin HV. Cross-reactivity of thirteen monoclonal antibodies with ten vaccinia cDNA expressed rat, mouse and human cytochrome P450s. Biochem Pharmacol 1993;46:787–90.
[42] Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979;18:5294–9.
[43] Kocarek TA, Schuetz EG, Guzelian PS. Differentiated induction of cytochrome P450b/e and P450p mRNAs by a dose of phenobarbital in primary cultures of adult rat hepatocytes. Mol Pharmacol 1990;38:440–4.

[44] Yanev S, Kent UM, Pandova B, Hollenberg PF. Selective mechanism-based inactivation of cytochromes P-450 2B1 and P-450 2B6 by a series of xanthates. Drug Metab Dispos 1999;27:600–4.
[45] Koenigs LL, Trager WF. Mechanism-based inactivation of cytochrome P450 2B1 by 8-methoxypsoralen and several other furancoumarins. Biochemistry 1998;37:13184–93.
[46] Connors GJ, Tarbox AR, McLaughlin EJ. Contrasting binge and continuous alcoholic drinkers using demographic and drinking history variables. Alcohol Alcohol 1986;21:105–10.
[47] Lieber CS, DeCarli LM. Hepatic microsomal ethanol-oxidizing system. *In vitro* characteristics and adaptive properties *in vivo*. J Biol Chem 1970;245:2505–12.
[48] Bernal CA, Vazquez JA, Adibi SA. Liver triglyceride concentration and body protein metabolism in ethanol-treated rats: effect of energy and nutrient supplementation. Gastroenterology 1992;103:289–95.
[49] Longo V, Ingelman-Sundberg M. Acetone-dependent regulation of cytochromes P450 2E1 and P450 2B1 in rat nasal mucosa. Biochem Pharmacol 1993;46:1945–51.
[50] Ibach B, Appel K, Gebicke-Haerter P, Meyer RP, Friedberg T, Knoth R, Volk B. Effect of phenytoin on cytochrome P450 2B mRNA expression in primary rat astrocyte cultures. J Neurosci Res 1998;54: 402–11.
[51] Nanji AA, Zhao S, Lamb RG, Dannenberg AJ, Sadrzadeh SM, Waxman DJ. Changes in cytochromes P450 2E1, 2B1, 4A, and phospholipases A and C in the intragastric feeding rat model for alcoholic liver disease: relationship to dietary fats and pathologic liver injury. Alcohol Clin Exp Res 1994;18:902–8.
[52] Adir J, Wildfeuer W, Miller RP. Effect of ethanol pretreatment on the pharmacokinetics of nicotine in rats. J Pharmacol Exp Ther 1980; 212:274–9.
[53] Hammond DK, Bjerke RJ, Langone JJ, Strobel HW. Metabolism of nicotine by rat liver cytochromes P-450: assessment utilizing monoclonal antibodies to nicotine and cotinine. Drug Metab Dispos 1991; 19:804–8.
[54] Benowitz NL, Jones RT, Jacob P. Additive cardiovascular effects of nicotine and ethanol. Clin Pharmacol Ther 1986;40:420–4.
[55] Strobel S, Halpert JR. Reassessment of cytochrome P450 2B2: catalytic specificity and identification of four active site residues. Biochemistry 1997;36:11697–706.
[56] Garro AJ, Espina N, Lieber CS. Alcohol and cancer. Alcohol Health Res World 1992;16:81–6.
[57] Garro AJ, Lieber CS. Alcohol and cancer. Annu Rev Pharmacol Toxicol 1990;30:219–49.
[58] McKenry LM, Salerno E. Pharmacology in nursing. St. Louis: Mosby Year Book, 1992.
[59] Hardwick JP, Gonzalez FJ, Kasper CB. Transcriptional regulation of rat liver epoxide hydratase, NADPH-cytochrome P-450 oxidoreductase, and cytochrome P-450b genes by phenobarbital. J Biol Chem 1983;258:8081–5.
[60] Stoltz C, Vachon MH, Trottier E, Dubois S, Paquet Y, Anderson A. The CYP2B2 phenobarbital response unit contains an accessory factor element, and a putative glucocorticoid response element essential for conferring maximal phenobarbital responsiveness. J Biol Chem 1998;273:8528–36.
[61] Honkakoski P, Zelko I, Sueyoshi T, Negishi M. The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. Mol Cell Biol 1998;18:5652–8.
[62] Kawamoto T, Sueyoshi T, Zelko I, Moore R, Washburn K, Negishi M. Phenobarbital-responsive nuclear translocation of the receptor CAR in induction of the CYP2B gene. Mol Cell Biol 1999;19:6318–22.
[63] Eliasson E, Johansson I, Ingelman-Sundberg M. Ligand-dependent maintenance of ethanol-inducible cytochrome P-450 in primary rat hepatocyte cell cultures. Biochem Biophys Res Commun 1988;150: 436–43.
[64] Beresford T, Arciniegas D, Rojas D, Sheeder J, Teale P, Aasal R, Sandberg E, Reite M. Hippocampal to pituitary volume ratio: a specific measure of reciprocal neuroendocrine alterations in alcohol dependence. J Stud Alcohol 1999;60:586–8.
[65] Biewald J, Bromme HJ, Nilius R, Langner J. Effects of twelve months ethanol feeding on rat liver metabolic activity. Int J Mol Med 1999;3:279–84.
[66] Xu X, Ingram RL, Sonntag WE. Ethanol suppresses growth hormone-mediated cellular response in liver slices. Alcohol Clin Exp Res 1995;19:1246–51.
[67] Jarukamjorn K, Sakuma T, Miyaura J, Nemoto N. Different regulation of the expression of mouse hepatic cytochrome P450 2B enzymes by glucocorticoid and phenobarbital. Arch Biochem Biophys 1999;369:89–99.
[68] Donahue BS, Morgan ET. Effects of vanadate on hepatic cytochrome P450 expression in streptosotocin-diabetic rats. Drug Metab Dispos 1990;18:519–26.
[69] Sidhu JS, Omiecinski CJ. Insulin-mediated modulation of cytochrome 450 gene induction profiles in primary rat hepatocyte cultures. J Biochem Mol Toxicol 1999;13:1–9.
[70] Woodcroft KJ, Novak RF. Insulin differentially affects xenobiotic-enhanced, cytochrome P-450 (CYP)2E1, CYP2B, CYP3A, and CYP4A expression in primary cultured rat hepatocytes. J Pharmacol Exp Ther 1999;289:1121–7.
[71] De Waziers I, Garlatti M, Bouguet J, Beaune PH, Barouki R. Insulin down-regulates cytochrome P450 2B and 2E expression at the post-transcriptional level in the rat hepatoma cell line. Mol Pharmacol 1995;47:474–9.
[72] Yamazoe Y, Shimada M, Murayama N, Kato R. Suppression of levels of phenobarbital-inducible rat liver cytochrome P-450 by pituitary hormone. J Biol Chem 1987;262:7423–8.
[73] Shimada M, Murayama N, Nagata K, Hashimoto H, Ishikawa H, Yamazoe Y. A specific loss of growth hormone abolished sex-dependent expression of hepatic cytochrome P450 in dwarf rats: reversal of the profiles by growth hormone treatment. Arch Biochem Biophys 1997;337:34–42.
[74] Sharma MC, Agrawal AK, Sharma MR, Shapiro BH. Interactions of gender, growth hormone, and phenobarbital induction on murine Cyp2b expression. Biochem Pharmacol 1998;56:1251–8.