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Biochem Pharmacol. Author manuscript; available in PMC 2014 June 15.

Published in final edited form as:
Biochem Pharmacol. 2013 June 15; 85(12): 1848–1855. doi:10.1016/j.bcp.2013.04.014.

First demonstration that brain CYP2D-mediated opiate metabolic activation alters analgesia in vivo

Kaidi Zhou, MSc[a,b], **Jibran Y. Khokhar, PhD**[a,b,1], **Bin Zhao, PhD**[a,b], and **Rachel F. Tyndale, PhD**[a,b]

[a]Departments of Pharmacology & Toxicology and Psychiatry, University of Toronto, 1 King's College Circle, Toronto, Ontario, M5S 1A8, Canada

[b]Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, 250 College Street, Toronto, Ontario, M5T 1R8, Canada

[1]Department of Psychiatry, Dartmouth College, One Medical Center Drive, Lebanon, New Hampshire, 03756, United States

Abstract

The response to centrally-acting drugs is highly variable between individuals and does not always correlate with plasma drug levels. Drug-metabolizing CYP enzymes in the brain may contribute to this variability by affecting local drug and metabolite concentrations. CYP2D metabolizes codeine to the active morphine metabolite. We investigate the effect of inhibiting brain, and not liver, CYP2D activity on codeine-induced analgesia. Rats received intracerebroventricular injections of CYP2D inhibitors (20 μg propranolol or 40 μg propafenone) or vehicle controls. Compared to vehicle-pretreated rats, inhibitor-pretreated rats had: a) lower analgesia in the tail-flick test ($p<0.05$) and lower areas under the analgesia-time curve ($p<0.02$) within the first hour after 30 mg/kg subcutaneous codeine, b) lower morphine concentrations and morphine to codeine ratios in the brain ($p<0.02$ and $p<0.05$, respectively), but not in plasma ($p>0.6$ and $p>0.7$, respectively), tested at 30 min after 30 mg/kg subcutaneous codeine, and c) lower morphine formation from codeine *ex vivo* by brain membranes ($p<0.04$), but not by liver microsomes ($p>0.9$). Analgesia trended toward a correlation with brain morphine concentrations ($p=0.07$) and correlated with brain morphine to codeine ratios ($p<0.005$), but not with plasma morphine concentrations ($p>0.8$) or plasma morphine to codeine ratios ($p>0.8$). Our findings suggest that brain CYP2D affects brain morphine levels after peripheral codeine administration, and may thereby alter codeine's therapeutic efficacy, side-effect profile and abuse liability. Brain CYPs are highly variable due to genetics, environmental factors and age, and may therefore contribute to interindividual variation in the response to centrally-acting drugs.

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Corresponding author: Dr. Rachel F. Tyndale Room 4326, Medical Sciences Building, 1 King's College Circle Toronto, Ontario M5S 1A8 Phone: 416-978-6374 Fax: 416-978-6395 r.tyndale@utoronto.ca.
kaidi.zhou@mail.utoronto.caJibran.Y.Khokhar@Dartmouth.eduzhao.bin@utoronto.ca.

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CONFLICT OF INTEREST
Dr. Rachel Tyndale has participated in one day advisory meetings for Novartis and McNeil.

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Keywords

cytochrome P450; codeine; analgesia; drug metabolism; pharmacokinetics; neuropharmacology

1. INTRODUCTION

Cytochromes P450 (CYPs) are drug-metabolizing enzymes which are primarily expressed in the liver but are also expressed in other tissues such as the brain. CYPs in the brain are active *in vivo* [1], and in some cell types, brain CYPs are expressed at levels as high as those in the liver [2]. There is large interindividual variation in the response to centrally-acting drugs, which does not always correlate with plasma drug levels [3]. This may be caused, in some circumstances, by variation in the degree of metabolism by brain CYPs, which may affect local drug and metabolite levels in the brain, and in turn influence drug response.

Cytochrome P450 2D6 (CYP2D6) metabolizes many centrally-acting drugs, including clinically prescribed drugs (e.g. risperidone, fluoxetine, codeine) as well as drugs of abuse (e.g. amphetamine, 3,4-methylenedioxymethamphetamine) [4]. Brain CYP2D6 levels are influenced by multiple factors. *CYP2D6* is genetically polymorphic, which results in different phenotypes ranging from poor metabolizers, who lack CYP2D6 function, to ultrarapid metabolizers, who have multiple functional copies of *CYP2D6* [5]. Levels of brain CYP2D6, unlike hepatic CYP2D6, are induced by drugs; for example, they are higher in smokers and alcoholics [6-8] and in animals exposed to nicotine and ethanol [7,9,10]. Brain CYP2D6 levels also increase with age while hepatic CYP2D6 levels remain the same [11,12]. Therefore, genetics, environmental inducers and age may contribute to variation in brain CYP2D6 expression and activity, and thereby alter the metabolism of, and response to, centrally-acting drugs.

CYP2D6 metabolizes codeine to the active morphine metabolite [13]. Since morphine has a 3000-fold greater affinity for the mu-opioid receptor than does codeine [14], analgesia from codeine depends on its conversion to morphine. Thus, *CYP2D6* poor metabolizers, and individuals pretreated with the CYP2D6 inhibitor quinidine, produce no morphine from codeine and experience no analgesia [15-17]. Codeine is metabolized to morphine mainly in the liver; morphine then crosses into the brain where it can interact with mu-opioid receptors to elicit analgesia. However, morphine is less permeable across the blood-brain barrier (BBB) than codeine, and is also actively transported out of the brain by efflux transporters [18]; therefore, there is a delay in morphine's entry into the brain compared to codeine's entry [19]. In rats given peripheral injections of either codeine or morphine, where the doses of the two drugs were adjusted to give the same plasma morphine levels at 30 min after injection, brain morphine was only found in codeine- and not in morphine-treated rats, suggesting that at 30 min, morphine had not yet crossed into the brain from the periphery [20]. Therefore, the morphine found in the brain during the first 30 min after codeine injection may be due to local codeine metabolism in the brain.

Our objective was to examine the role of rat brain CYP2D in the metabolic activation of codeine and subsequent analgesia. The rat is a useful model of human CYP2D6-mediated drug metabolism since rat CYP2D has similar substrate specificity and enzymatic activity to human CYP2D6 [21]. The role of brain, as opposed to hepatic, CYP2D was investigated using intracerebroventricular (i.c.v.) injection of CYP2D inhibitors in rats, which inhibited brain CYP2D activity while leaving hepatic CYP2D activity unchanged. We hypothesized that compared to vehicle pretreatment, CYP2D inhibitor pretreatment would result in lower brain morphine levels and therefore lower analgesia after subcutaneous codeine injection. Clarifying the impact of brain CYP2D-mediated metabolism on codeine response will help

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us understand the potential role brain CYPs have in interindividual variation in the response to centrally-acting drugs.

2. MATERIALS AND METHODS

2.1 Animals

Male adult Wistar rats (250–300 g; Charles River, St-Constant, QC, Canada) were kept in pairs or triplets under a 12 h artificial light/dark cycle. Rats were handled daily to acclimate them to testing procedures. All procedures were approved by the Animal Care Committee at the University of Toronto.

2.2 Drugs

Propranolol hydrochloride (Sigma-Aldrich, Oakville, ON, Canada), a CYP2D mechanism-based inhibitor [22], was dissolved in artificial cerebrospinal fluid (ACSF; 126 mM NaCl, 2.68 mM KCl, 1 mM Na_2HPO_4, 0.88 mM $MgSO_4$, 22 mM $NaHCO_3$, 1.45 mM $CaCl_2$, 11 mM D-glucose; pH 7.4), and 20-40 μg of the base was injected i.c.v. in a 1-4 μl volume. Propranolol undergoes 4-hydroxylation by CYP2D which is associated with the formation of a reactive metabolite in the active site of the enzyme [22]. This reactive species covalently binds to the active site, thereby inactivating the enzyme, and remains bound even after microsome and membrane preparation [23,24]. Propafenone hydrochloride (Sigma-Aldrich), a CYP2D competitive inhibitor [25], was dissolved in a 20% w/v solution of 2-hydroxypropyl-β-cyclodextrin (cyclodextrin; Sigma-Aldrich) in water, and 40 μg of the base was given i.c.v. in a 4 μl volume. The inhibitor doses were chosen based on pilot studies showing that they did not inhibit hepatic CYP2D.

Codeine phosphate (PCCA, London, ON, Canada) was dissolved in saline (0.9% NaCl; pH 7) and injected subcutaneously (s.c.) at 30 mg base/kg body weight. This dose was chosen based on a previous study which showed that it induced analgesia in the tail-flick test in all animals tested [26]. Morphine sulfate (PCCA) was dissolved in distilled water and injected s.c. at 5 mg base/kg body weight. This dose was chosen as it produces equivalent analgesia in the tail-flick test to the 30 mg/kg codeine dose used [27].

2.3 Inhibition of rat brain CYP2D

Rats were anesthetized with isoflurane and placed in a stereotaxic frame. Intracerebroventricular cannulation was performed on rats that were to be tested for analgesia, and in rats that were to receive propafenone and subsequently be tested for *in vivo* codeine metabolism. Intracerebroventricular cannulas were inserted into the right lateral ventricle (Bregma coordinates: dorsal-ventral, -3.6; anterior-posterior, -0.9; lateral, -1.4) [28]. After a one week recovery period, rats received an i.c.v. injection into the cannula of either 40 μg (for control experiments) or 20 μg (for all other experiments) propranolol dissolved in 4 μl ACSF, 4 μl ACSF, 40 μg propafenone dissolved in 4 μl cyclodextrin, or 4 μl cyclodextrin.

Rats that were to receive propranolol and then be tested for *in vivo* codeine metabolism or *ex vivo* CYP2D activity received an i.c.v. injection into the right lateral ventricle (same coordinates as above) of either 20 μg propranolol dissolved in 1 μl ACSF, or 1 μl ACSF.

2.4 Analgesia

The heat beam of the tail-flick meter (Columbus Instruments, Columbus, OH, United States) was placed 2 to 3 cm from the end of the tail. Heat intensity was adjusted to produce baseline tail-flick latencies (TFLs) of ~2-4 sec, and a cut-off of 10 sec was used to avoid damaging the tail skin. In drug-naïve rats, TFL was measured three times in each rat, and the

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mean was used as that rat's baseline TFL. Codeine or morphine was injected s.c. 24 h after i.c.v. injection of propranolol or ACSF, or 5 min after i.c.v. injection of propafenone or cyclodextrin, and TFL was measured for the next 2 h. Analgesia was expressed as percentage of maximal analgesic effect (%MPE): %MPE = (test TFL – baseline TFL) / (cut-off – baseline TFL)

Test TFLs lower than baseline TFL were given a %MPE of zero. Area under the analgesia-time curve was determined by the linear trapezoid rule using PK Functions for Microsoft Excel (J. I. Usansky, A. Desia, D. Tan-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergon, Irvine, CA). A within-animal design was used in which, after a 2 week washout period, rats were crossed over (inhibitor vs. vehicle) and retested with opioid. In both phases, half of the rats received inhibitor and the other half received vehicle. TFL was also measured 24 h after propranolol injection and 5 min after propafenone injection in the absence of opioid.

2.5 Codeine and morphine levels in brain and plasma

Rats received s.c. codeine 24 h after i.c.v. propranolol or ACSF injection, or 5 min after i.c.v. propafenone or cyclodextrin injection. Propranolol-pretreated rats were decapitated at 30, 60 or 90 min after codeine injection, and propafenone-pretreated rats were decapitated at 30 min after codeine injection. Blood was centrifuged at 5000 g for 10 min. Brains were homogenized 1:3 (w/v) in 0.01 M HCl and centrifuged at 5000 g for 10 min [29]. Morphine and codeine concentrations were measured using HPLC as described below.

2.6 Membrane and microsome preparation

Brain CYPs are found in the microsomal membranes, but also in plasma membranes and mitochondrial membranes [21,30-32]. Rat CYP2D activity has been detected in microsomal, plasma, mitochondrial and nuclear membranes [30]. Therefore, total membrane fractions were prepared. From rats that received an i.c.v. injection of propranolol or ACSF 24 h prior to sacrifice, brains were homogenized in 10 ml ACSF and centrifuged at 3000 g for 5 min. The pellet was resuspended in 10 ml ACSF and centrifuged at 3000 g for 5 min. The two supernatants were combined and centrifuged at 100 000 g for 60 min, and the resulting pellet was resuspended in ACSF [30]. Liver microsomes were prepared as done previously [33]. Protein concentrations were measured using the Bradford assay with a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Mississauga, Canada).

2.7 *Ex vivo* codeine oxidation

For brain codeine oxidative metabolism, freshly prepared membranes (6 mg protein/ml) were incubated with 500 μM (10 x Km) [34] codeine, 5 mM $MgCl_2$ and 1 mM NADPH in ACSF (pH 7.4) for 120 min at 37°C under 95% O_2/5% CO_2 in a final volume of 1 ml. The reaction was stopped with 200 μl bicarbonate buffer solution (1 M, pH 9.7). For hepatic codeine oxidative metabolism, microsomes (0.25 mg protein/ml) were incubated with 500 μM codeine, 5 mM $MgCl_2$ and 1 mM NADPH in 100 mM NaH_2PO4*H_2O buffer (pH 7.4) for 20 min at 37°C in a final volume of 0.5 ml [35]. The reaction was stopped with 100 μl acetonitrile. Controls included conditions without substrate and without membrane protein.

2.8 HPLC

2.8.1 Sample preparation—HPLC methods were based on modifications to previous techniques [36,37]. For brain samples from *in vivo* and *ex vivo* metabolism studies and plasma samples from *in vivo* metabolism studies, 1 μg 2-benzoxazolinone (internal standard) was added. Solid phase extraction was performed as done previously [36]. The eluate was evaporated to dryness at 37°C under a nitrogen stream and reconstituted with 110

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μl of mobile phase and 100 μl of the solution was injected. For liver samples from *ex vivo* metabolism studies, after stopping the incubation reaction, samples were centrifuged at 13 250 g for 10 min and 90 μl of the supernatant was injected [34,38].

2.8.2 Chromatographic conditions—Samples were analyzed by HPLC with ultraviolet detection (Agilent 1200 Separation Module). Detection was at 214 nm and morphine, codeine and internal standard were separated on an Agilent ZORBAX SB-C18 Column (250 x 4.6 mm I.D.; particle size, 5 μm). The mobile phase used was methanol-0.05M phosphate buffer, pH 5.8 (29.3/70.7, v/v) and the flow rate was 1 ml/min. The retention times were 4.1 min for morphine, 9.4 min for codeine, and 16.7 min for the internal standard. For plasma samples from *in vivo* metabolism studies, as well as for liver and brain samples from *ex vivo* metabolism studies, the limits of quantification and extraction efficiency respectively were 25 ng/ml and 76.9% for morphine and 250 ng/ml and 83.5% for codeine. For brain samples from *in vivo* metabolism studies, the limits of quantification and extraction efficiency respectively were 5 ng/g and 82.7% for morphine and 50 ng/g and 90.1% for codeine.

2.9 Statistical analyses

Paired t-tests were used for within-animal comparisons. Unpaired t-tests were used for between-animal comparisons.

3. RESULTS

3.1 Inhibition of brain CYP2D reduced codeine-induced analgesia

We examined whether inhibiting brain CYP2D would reduce analgesia following codeine administration. Compared to vehicle pretreatment, 20 μg i.c.v. propranolol pretreatment resulted in significantly lower %MPE at 15 ($p<0.04$), 20 ($p<0.02$), 30 ($p<0.006$) and 40 ($p<0.005$) min after 30 mg/kg s.c. codeine injection (Fig. 1A). Compared to vehicle pretreatment, 40 μg i.c.v. propafenone pretreatment resulted in significantly lower %MPE at 20 ($p<0.03$), 30 ($p<0.03$) and 40 ($p<0.05$) min after 30 mg/kg s.c. codeine injection (Fig. 1E).

Compared to vehicle pretreatment, 20 μg i.c.v. propranolol pretreatment resulted in significantly lower area under the analgesia-time curve (AUC) for 0-30 ($p<0.009$) and 0-60 ($p<0.02$) min after 30 mg/kg s.c. codeine injection (Fig. 1, B and C). Likewise, 40 μg i.c.v. propafenone pretreatment resulted in significantly lower AUC for 0-30 ($p<0.01$) and 0-60 ($p<0.02$) min after codeine injection (Fig. 1, F and G). Neither inhibitor resulted in lower AUC for 60-120 min after codeine injection (propranolol: $p>0.9$, Fig. 1D; propafenone: $p>0.3$, Fig. 1H).

We assessed whether either of the two CYP2D inhibitors on their own would have an effect on nociception. There was no significant difference between baseline TFL [3.20+0.12 sec (mean+SEM)] and TFL 24 h after 40 μg i.c.v. propranolol pretreatment [3.37+0.16 sec (mean+SEM); n=10; $p>0.3$ using a paired t-test]. There was also no significant difference between baseline TFL [2.96+0.12 sec (mean+SEM)] and TFL 30 min after 40 μg i.c.v. propafenone pretreatment [2.75+0.23 sec (mean+SEM); n=8; $p>0.1$ using a paired t-test].

3.2 Inhibiting brain CYP2D did not affect morphine-induced analgesia

Morphine (5 mg/kg s.c.) gave a similar level of analgesia as observed after 30 mg/kg s.c. codeine [The 0-120 min AUCs (%MPE*min) were as follows. Codeine with ACSF (n=16): 5179+966 (mean+SEM), morphine with ACSF (n= 12): 5344+950 (mean+SEM), $p>0.7$ using an unpaired t-test; codeine with cyclodextrin (n=12): 7748+1057 (mean+SEM), morphine with cyclodextrin (n=6): 7425+1070 (mean+SEM), $p>0.8$ using an unpaired t-

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test]. Since morphine is the active analgesic compound, and it is not metabolized by CYP2D, its analgesic effect should not be altered by changes in CYP2D activity. Compared to vehicle pretreatment, 40 μg i.c.v. propranolol pretreatment did not result in significantly different %MPE ($p>0.06$ at all time points; Fig. 2A) or AUC ($p>0.4$ for all time periods; Fig. 2, B-D) after 5 mg/kg s.c. morphine injection. Compared to vehicle pretreatment, 40 μg i.c.v. propafenone pretreatment did not result in significantly different %MPE ($p>0.09$ at all time points; Fig. 2E) or AUC ($p>0.2$ for all time periods; Fig. 2, F-H) after 5 mg/kg s.c. morphine injection.

3.3 Inhibitor-pretreated rats had lower morphine levels in the brain, but not the plasma, at 30 min after codeine injection

To assess the pharmacokinetic effects of the CYP2D inhibitors and to see if these are consistent with differences in analgesia, we examined codeine and morphine levels in brain and plasma at 30 min after codeine injection, when the largest difference in analgesia occurred between inhibitor- and vehicle-preteatment (Fig. 1, A and E). Compared to vehicle-pretreated rats, 20 μg i.c.v. propranolol-pretreated rats had significantly lower morphine concentrations and morphine to codeine ratios in the brain ($p<0.02$, $p<0.05$, respectively) but equal concentrations and ratios in plasma ($p>0.6$, $p>0.7$, respectively) (Fig. 3, A-D). Compared to vehicle-pretreated rats, 40 μg i.c.v. propafenone-pretreated rats also had significantly lower morphine concentrations and morphine to codeine ratios in the brain ($p<0.006$, $p<0.03$, respectively) but equal concentrations and ratios in plasma ($p>0.6$, $p>0.8$, respectively) (Fig. 3, E-H). Percentage of maximal analgesic effect correlated with brain morphine concentration ($p=0.07$, Fig. 4A) and brain morphine to codeine ratio ($p<0.005$, Fig. 4C). There was no correlation between %MPE and plasma morphine concentration ($p>0.8$, Fig. 4B) or plasma morphine to codeine ratio ($p>0.8$, Fig. 4D).

At both 60 and 90 min after 30 mg/kg s.c. codeine injection, there was no significant difference in brain morphine concentrations ($p>0.1$, $p>0.9$, respectively; Fig. 5A) or brain morphine to codeine ratios ($p>0.4$, $p>0.2$, respectively; Fig. 5B) between 20 μg i.c.v. propranolol- and vehicle-pretreated rats.

3.4 Inhibiting brain CYP2D *in vivo* lowered *ex vivo* codeine metabolism in the brain but not liver

In rats pretreated *in vivo* with 20 μg i.c.v. propranolol and sacrificed 24 h later, morphine formation from codeine *ex vivo* in brain membranes was significantly lower than in vehicle-pretreated rats ($p<0.04$; Fig. 6A). In liver microsomes, there was no significant difference in morphine formation between propranolol- and vehicle-pretreated rats ($p>0.9$; Fig. 6B).

4. DISCUSSION

This is the first study to show that brain CYP2D-mediated metabolism can alter the effect of a centrally-acting drug. Inhibiting brain CYP2D decreased the first 60 min of codeine-induced analgesia, but did not alter baseline nociception or morphine-induced analgesia. This confirms that the inhibitors did not lower codeine-induced analgesia by having their own effects on nociception or by interfering with morphine's mechanisms of action. Furthermore, showing that analgesia from codeine was reduced by two CYP2D inhibitors with different structures, different mechanisms of inhibition (propranolol is mechanism-based [22], propafenone is competitive [25]) and different pharmacological actions supports that our results were indeed due to brain CYP2D inhibition as opposed to another effect of either inhibitor.

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At 30 min after codeine injection, inhibitor-pretreated rats had lower morphine levels in the brain but not in the plasma, suggesting that inhibitor pretreatment reduced codeine metabolism by the brain but not by the liver. Analgesia correlated with brain morphine levels, but not with plasma morphine levels, suggesting that analgesia is mediated by morphine in the brain and that the differences in codeine-induced analgesia we observed after inhibitor versus vehicle pretreatment were due to differences in brain morphine levels. At 60 and 90 min after codeine injection, when there was no longer a difference in analgesia after inhibitor versus vehicle pretreatment, there was correspondingly no difference in brain morphine levels.

As shown in Fig. 2, morphine-injected rats experienced analgesia within the first 30 min, indicating that morphine was able to enter the brain by this time. This is in contrast to the previously mentioned study in which morphine was undetectable in the brain at 30 min after injection [20], which is likely due to the use of a higher morphine dose in the current study and to the difference in route of administration (intraperitoneal versus subcutaneous used here) altering the relative levels of morphine and morphine glucuronides (which are less permeable across the BBB [39]).

We did not have complete inhibition of CYP2D in the brain, as indicated by *ex vivo* CYP2D activity from brain membranes of inhibitor-pretreated rats. This may explain why inhibitor pretreatment did not result in significantly lower analgesia than vehicle pretreatment at very early time points. Still, morphine formation from codeine was lower in the brain membranes but not liver microsomes of inhibitor-pretreated rats, indicating that inhibitor pretreatment reduced brain but not hepatic CYP2D activity, and that the lower brain morphine levels seen in inhibitor-pretreated rats were indeed due to reduced brain CYP2D-mediated metabolism of codeine to morphine. Therefore, while hepatic CYP2D may be responsible for the bulk of morphine formed from systemic codeine injection, *in situ* codeine metabolism in the brain can meaningfully impact brain morphine levels and, in turn, the response to codeine.

Together our results suggest the following description of codeine metabolism in the brain and liver and the resulting analgesia. After codeine administration, analgesia is initially mediated mainly by morphine formed by brain CYP2D, as relatively little morphine formed by hepatic CYP2D has crossed into the brain yet. Thus, lower (or higher) brain CYP2D activity and the consequent decrease (or increase) in brain morphine levels results in lesser (or greater) analgesia. Over time, morphine generated by hepatic CYP2D accumulates in the periphery and crosses into the brain; because more morphine is formed by the liver than by the brain, analgesia at this later time period is mediated mainly by morphine formed by hepatic CYP2D that enters the brain. Our interpretation is supported by the findings that morphine could be detected in the brains of codeine- but not morphine-injected rats at 30 min after intraperitoneal injection [20], brain uptake of codeine is faster than that of morphine [19], morphine is transported out of the brain by efflux transporters [18], and the fact that morphine has one less methyl group than codeine, which is expected to make morphine less lipid soluble and therefore less able to cross the BBB. Altogether, our findings suggest that brain CYP2D is responsible for analgesia during the initial period after codeine administration, and that variation in brain CYP2D activity may influence the onset of analgesia from codeine.

The clinical implications of our findings are that individuals with higher brain CYP2D6 activity (smokers, alcoholics, older people, CYP2D6 ultrarapid metabolizers) may experience a quicker onset of analgesia from codeine, whereas those with lower brain CYP2D6 activity (non-smokers, non-alcoholics, individuals taking centrally-acting CYP2D6 inhibitors, CYP2D6 intermediate metabolizers) may experience a slower onset of analgesia, although, to our knowledge, this has not been explicitly tested yet. The latter individuals,

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because of their potential delay in pain-relief, may re-dose on codeine and experience increased side effects or toxicity when the large amounts of morphine from the periphery finally enter the brain. Those with high CYP2D6 activity are also susceptible to codeine toxicity. For example, when a *CYP2D6* ultrarapid metabolizer received a small dose of codeine, this lead to serious toxicity as a result of the high levels and fast rates of morphine formed [40]. Our observation that brain CYP2D activity influences the early analgesic effects of codeine also has implications for codeine's abuse liability. The reinforcing qualities of codeine come from its morphine metabolite [41,42]. Therefore, the initial reinforcing effects of codeine should be mediated by its metabolism to morphine in the brain, before morphine from the periphery has crossed into the brain. Since the formation of morphine in the brain should occur at a lower rate in those with decreased brain CYP2D6 activity, these individuals are expected to experience a slower onset of reinforcing effects from codeine and may be less prone to codeine abuse. In support of this, *CYP2D6* poor metabolizers are underrepresented among individuals dependent on oral opioid drugs [41], individuals pretreated with CYP2D6 inhibitors experienced fewer positive subjective effects from codeine [42], and long-term users of codeine who were treated a CYP2D6 inhibitor had a decrease in opioid use [43]. Therefore, variation in brain CYP2D6 activity due to genetics, environmental inducers or age may lead to differences in brain morphine levels after codeine administration, which may have implications for the clinical utility and abuse liability of codeine.

Our findings that brain CYP2D plays a significant role in the metabolism and effect of codeine suggest that brain CYP2D6 may also have an important impact on the response to the numerous other centrally-acting drugs metabolized by CYP2D6. For example, desipramine, an antidepressant inactivated by CYP2D6, was less effective in older (>75 years) individuals than in younger patients when controlling for drug dose and plasma levels [44]. The increased levels of brain (and not liver) CYP2D6 in individuals 60 to 80 years of age [11] may increase rates of desipramine inactivation within the brain and contribute to its reduced efficacy. CYP2D6 metabolizes various antipsychotics including risperidone [45]. Plasma levels of risperidone and its active metabolite do not correlate with extrapyramidal side effects (EPS) [46,47], and this may be because of variation in drug metabolism in the brain. Smokers have less EPS from antipsychotics than non-smokers [48], and this may be due to their elevated brain (and not liver) CYP2D6 levels [6,7] which may increase the clearance of antipsychotics [49]. Higher brain CYP2D6 activity can also be speculated to be a risk factor for the abuse of recreational drugs that are activated (e.g. dextromethorphan) or inactivated by CYP2D6 (e.g., amphetamine), as the abuse liability of a drug depends partly on the quickness of onset and offset of its reinforcing effects [50,51]. In sum, variation in brain CYP2D6 levels may alter the therapeutic efficacy, abuse liability and/or side effect profile of multiple clinical drugs, and this remains to be investigated.

Our data suggest that other brain CYPs may also be functional *in vivo* and have an important role in local substrate metabolism. This has been demonstrated to be the case with CYP2B. Manipulating rat CYP2B activity in the brain (and not liver) altered the sleep times induced by the anesthetic propofol, which is metabolically inactivated by CYP2B [52], and the neurotoxicity from chlorpyrifos, which is metabolically activated by CYP2B [53]. Therefore, variation in other drug-metabolizing CYPs in the brain may also alter response to centrally-acting drugs and toxins.

In conclusion, we found that inhibiting rat brain CYP2D decreased the metabolism of codeine to morphine in the brain and reduced codeine-induced analgesia. Our results reveal that rat brain CYP2D is functional *in vivo* and plays a significant role in the metabolism and effect of codeine, suggesting that brain CYP2D may have an important impact on the response to the numerous other centrally-acting CYP2D substrates. Differences in human

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brain CYP2D6 activity, whether due to genetic variation, exposure to alcohol or nicotine, or age, may contribute to the interindividual variation in therapeutic efficacy, side effect profiles and abuse liability of centrally-acting drugs metabolized by CYP2D6. The expression of multiple families of CYPs in the brain, and their diverse range of centrally-acting substrates, supports the brain as being an important organ in drug metabolism.

Acknowledgments

The authors would like to thank Dr. Sharon Miksys for her technical assistance and sharing of knowledge and advice, and Steven Lo for his technical assistance. This work was supported by a Canadian Research Chair (RFT), the Endowed Chair in Addiction for the Department of Psychiatry (RFT), Canadian Institutes of Health Research (grant number MOP97751), National Institutes of Health (grant number DA 02083), Centre for Addiction and Mental Health and the Centre for Addiction and Mental Health Foundation, the Canada Foundation for Innovation (grant numbers 20289, 16014), and the Ontario Ministry of Research and Innovation.

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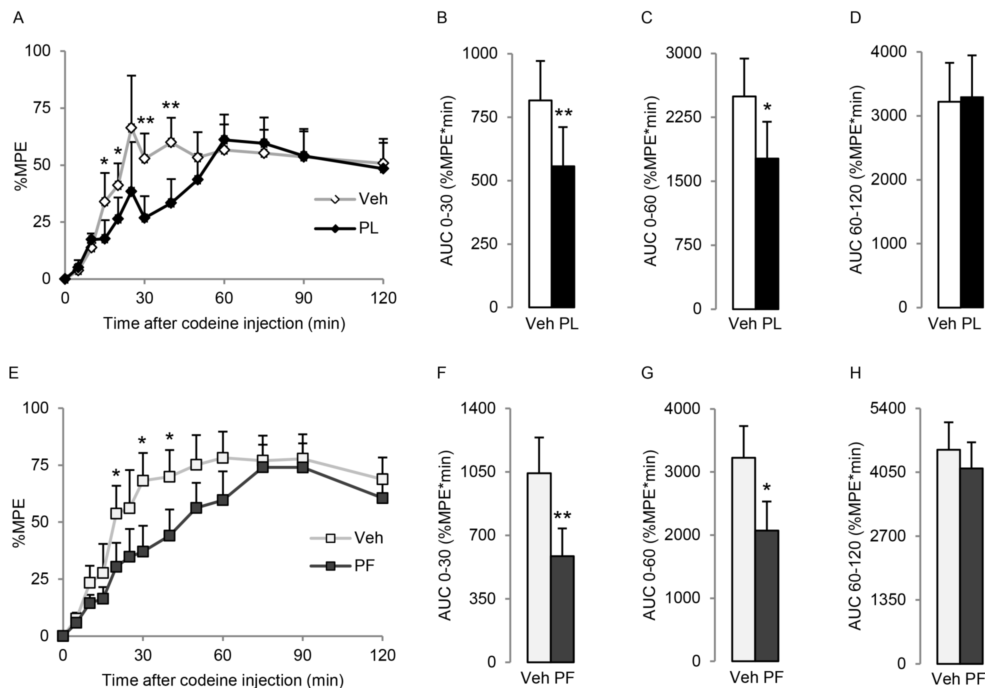

Figure 1.

Inhibiting brain CYP2D reduced the first 60 min of codeine-induced analgesia. Compared to vehicle pretreatment, propranolol pretreatment resulted in (A) significantly lower percentage of maximal analgesic effect (%MPE) at 15, 20, 30 and 40 min after codeine injection (n=4-16/time point), and significantly lower area under the analgesia-time curve (AUC) for (B) 0-30 min and (C) 0-60 min, but not for (D) 60-120 min or 0-120 min after codeine injection (n=16). Compared to vehicle pretreatment, propafenone pretreatment resulted in (E) significantly lower %MPE at 20, 30 and 40 min after codeine injection (n=7-12/time point), and significantly lower AUC for (F) 0-30 min and (G) 0-60 min, but not for (H) 60-120 min or 0-120 min after codeine injection (n=12). Error bars indicate SEM. *$p<0.05$, **$p<0.01$ using a paired t-test. Veh=vehicle, PL=propranolol, PF=propafenone.

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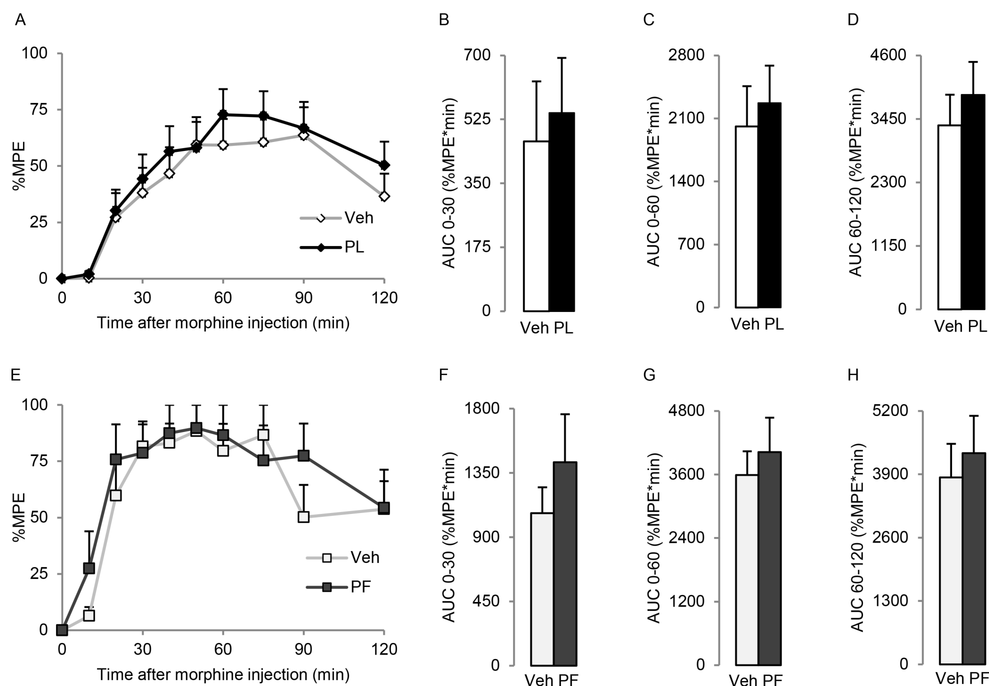

Figure 2.

Inhibiting brain CYP2D did not affect morphine-induced analgesia. Compared to vehicle pretreatment, propranolol pretreatment did not result in (A) significantly different percentage of maximal analgesic effect (%MPE) after morphine injection ($p>0.06$ at all time points, n=12), or significantly different area under the analgesia-time curve (AUC) for (B) 0-30 min, (C) 0-60 min, (D) 60-120 min or 0-120 min after morphine injection ($p>0.4$ for all time periods, n=12). Compared to vehicle pretreatment, propafenone pretreatment did not result in (E) significantly different %MPE after morphine injection ($p>0.09$ at all time points, n=6), or significantly different AUC for (F) 0-30 min, (G) 0-60 min, (H) 60-120 min or 0-120 min after morphine injection ($p>0.2$ for all time periods, n=6). Error bars indicate SEM. Paired t-tests were used. Veh=vehicle, PL=propranolol, PF=propafenone.

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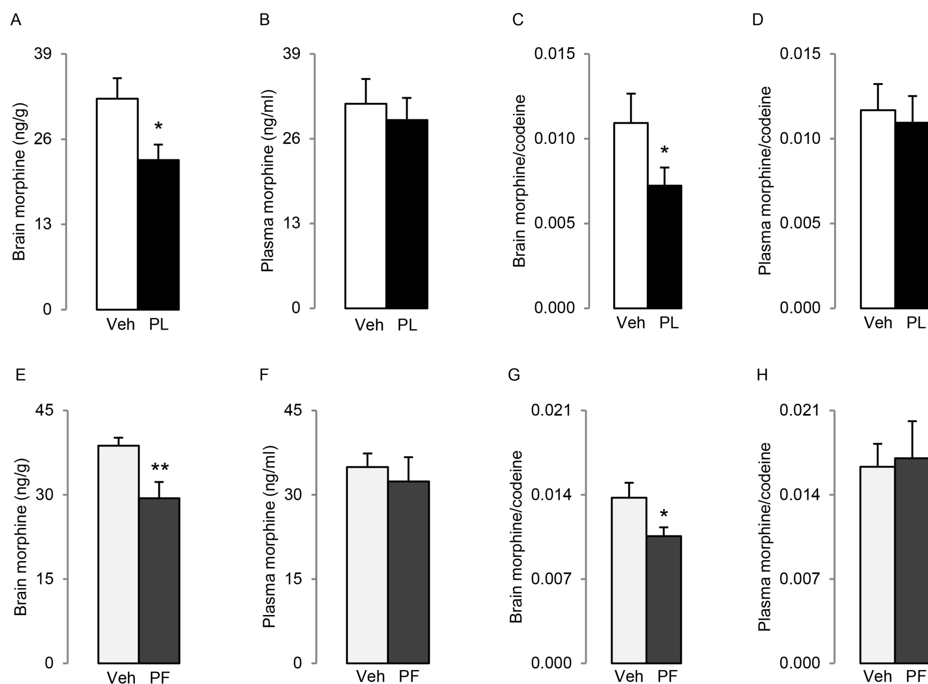

Figure 3.

Inhibitor-pretreated rats had lower morphine levels in the brain but not in plasma at 30 min after codeine injection. Compared to vehicle-pretreated rats, propranolol-pretreated rats had significantly lower morphine concentrations in (A) the brain but not in (B) plasma at 30 min after codeine injection (n=11/group), and significantly lower morphine to codeine ratios in (C) the brain but not in (D) plasma at 30 min after codeine injection (n=11/group). Compared to vehicle-pretreated rats, propafenone-pretreated rats had significantly lower morphine concentrations in (E) the brain but not in (F) plasma at 30 min after codeine injection (n=8/group), and significantly lower morphine to codeine ratios in (G) the brain but not in (H) plasma at 30 min after codeine injection (n=8/group). Error bars indicate SEM. *$p<0.05$, **$p<0.01$ using an unpaired t-test. Veh=vehicle, PL=propranolol, PF=propafenone.

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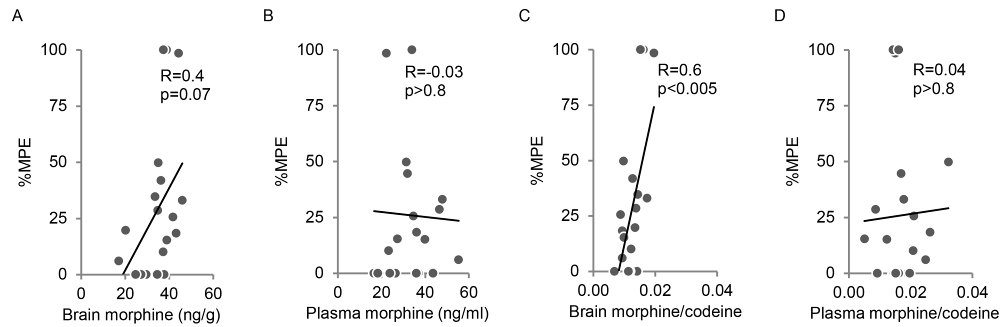

Figure 4.

Analgesia correlated with brain, and not plasma, morphine levels at 30 min after codeine injection. Percentage of maximal analgesic effect (%MPE) trended toward correlating with morphine concentration in (A) the brain (p=0.07, n=20) and not in (B) plasma (p>0.8, n=21). Percentage of maximal analgesic effect correlated significantly with morphine to codeine ratios in (C) the brain (p<0.004, n=20) and not in (D) plasma (p>0.8, n=21). Pearson correlation coefficients were used.

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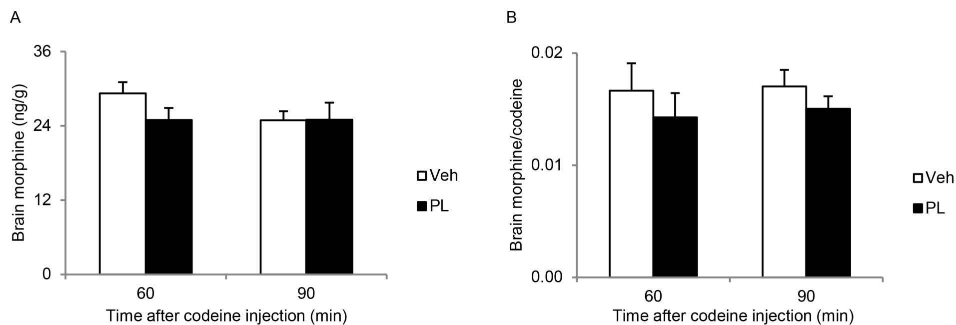

Figure 5.

Propranolol-pretreated rats did not have lower morphine levels in the brain at 60 or 90 min after codeine injection. At both 60 min (n=7/group) and 90 min (n=8/group) after codeine injection, there was no significant difference in (A) brain morphine concentrations ($p>0.1$, $p>0.9$, respectively) or (B) brain morphine to codeine ratios ($p>0.4$, $p>0.2$, respectively) between propranolol-pretreated rats and vehicle-pretreated rats. Error bars indicate SEM. Unpaired t-tests were used. Veh=vehicle, PL=propranolol.

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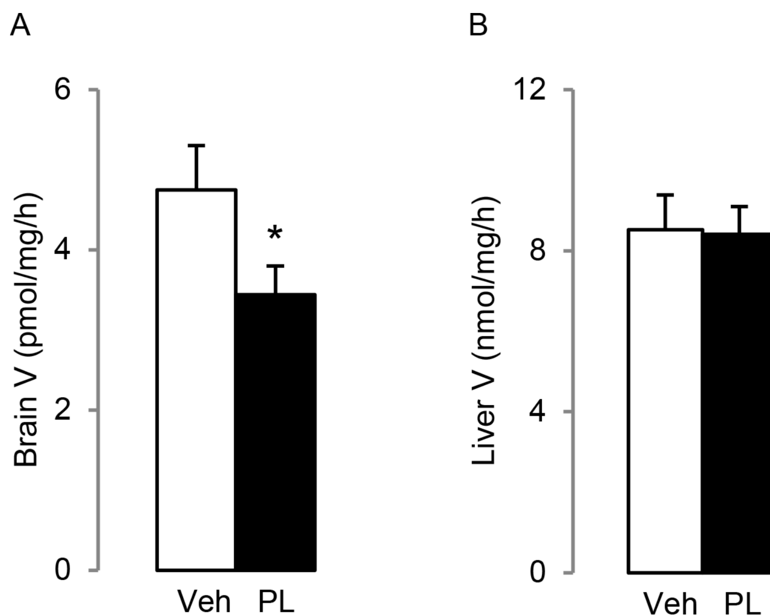

Figure 6.

Inhibiting brain CYP2D *in vivo* lowered *ex vivo* codeine metabolism to morphine by brain membranes but not by liver microsomes. (A) In brain membranes incubated with 500 μM codeine, velocity of morphine formation was significantly lower in propranolol-pretreated rats than in vehicle-pretreated rats (n=8/group). (B) In liver microsomes incubated with 500 μM codeine, there was no significant difference in the velocity of morphine formation between propranolol-pretreated rats and vehicle-pretreated rats (n=8/group). Error bars indicate SEM. *$p<0.05$ using an unpaired t-test. Veh=vehicle, PL=propranolol, V=velocity of morphine formation.