CYP2D activity in the brain acts independently from CYP2D in the liver to alter CNS drug levels and response.

Rachel Tyndale and Sharon Miksys, CAMH and University of Toronto

Drug metabolism in the Liver
Main organ for pharmacokinetics: alters drug levels and treatment response

Drug metabolism in the Brain:
CNS drug response and neurotoxicity
Rachel Tyndale has consulted for Apotex on material unrelated to the content of this presentation

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CYPs may metabolize drugs and neurotoxins locally in the brain

- Brain CYPs are active in vivo
- In some cell types, levels are as high as in hepatocytes
- Response to CNS-acting drugs does not always correlate with drug plasma levels

Miksys & Tyndale, 2009; Miksys et al., 2000; Michels & Marzuk, 1993
CYP enzymes in the Brain:
Understanding variation in drug and toxin response

Drug Activation
Codeine and CYP2D
Analgesia and Abuse Liability

Toxin Inactivation
MPP+ and CYP2D
Parkinson’s Disease
Haloperidol and CYP2D
Tardiff dyskinesia

Drug Inactivation
Propofol and CYP2B
Sedation
Nicotine and CYP2B
Self-Administration

Toxin Activation
Chlorpyrifos and CYP2B
Pesticide Neurotoxicity
Substrates of Polymorphic CYP2D6
Including clinical and non-clinical drugs, toxins and endogenous compounds

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Examples</th>
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<tbody>
<tr>
<td><strong>Opioids (e.g.)</strong></td>
<td>- Codeine</td>
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<tr>
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<td>- Hydrocodone</td>
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<td>- Oxycodone</td>
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<td>- Ethylmorphine</td>
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<td>- Theba ine</td>
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<td>- Dextromethorphan*</td>
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<td><strong>SSRIs (e.g.)</strong></td>
<td>- Fluoxetine</td>
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<td><strong>Antidepressants (e.g.)</strong></td>
<td>- Fluoxetine</td>
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<td>- Desipramine</td>
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<td>- Amitriptyline</td>
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<td>- Nortriptyline</td>
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<td>- Imipramine</td>
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<td>- Clomipramine</td>
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<td><strong>Misc.</strong></td>
<td>- Methoxyphenamine</td>
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<td>- Methylphenylpdate</td>
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<td>- Phencyclidine</td>
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<td><strong>Neuroleptics (e.g.)</strong></td>
<td>- Perhenazine</td>
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<td>- Thioridazine</td>
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<td>- Zuclopenthixol</td>
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<td><strong>Antiarrhythmics (e.g.)</strong></td>
<td>- Propafenone</td>
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<td>- Sparteine*</td>
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<td>- Flecainide</td>
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<td>- n-Propylajimaline</td>
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<td><strong>Beta Blockers (e.g.)</strong></td>
<td>- Metoprolol*</td>
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<td>- Timolol</td>
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<td><strong>Amphetamines (e.g.)</strong></td>
<td>- p-Methoxy-Amphetamine</td>
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<td>- Methamphetamine</td>
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<td>- Amphetamine</td>
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<td>- Methylene-dioxy-methamphetamine (MDMA, ecstasy)</td>
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<td><strong>Toxins</strong></td>
<td>- MPTP (demethylation, detoxification)</td>
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<td>- TIQ (hydroxylation, detoxification, less TIQ with chronic nicotine/smoke)</td>
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<td></td>
<td>- β Carbolines / 5-Methoxyindolethylamine (demethylation, detoxification)</td>
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<td>- Harmaline</td>
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<td>- Harmine</td>
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<td>- Pinoline</td>
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<td>- 5-methoxy-N,N-dimethyltryptamine</td>
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<tr>
<td><strong>Pesticides</strong></td>
<td>- parathion, diazinon, chlorpyrifos</td>
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<tr>
<td><strong>Endogenous / Neurotransmitters</strong></td>
<td>- Tyramine to Dopamine</td>
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<td>- Octamine to Norepinephrine</td>
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<td>- Synephrine to Epineprine</td>
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<td></td>
<td>- 5-Methoxytryptamine to serotonin</td>
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<td></td>
<td>- 21 OH’n of Progesterone</td>
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<td>- 21 OH’n of Allopregnanalone</td>
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</table>
Mouse brain CYP2D

Connie Chung, Frank Gonzalez, Sharon Miksys, Rachel Tyndale

Frontal Cortex

Hippocampus

20 μm

200 μm
CYP2D6 in Human Brain (Immunohistochemical Studies)

Hippocampus

Frontal Cortex

No 1° Antibody

Miksys et al., J Neurochem 2002
CYP2D6*4 splice site variant results in human brains without CYP2D6 protein

Miksys et al., J Neurochem 2002

13 Human Brain Regions

Density of CYP2D6 (+ S.D.)

Extensive metabolizers N=13

Poor metabolizers (*4/*4)

13 Human Brain Regions:
FC, TC, CG, OC, HC, EC, CD, PT, NA, GP, SN, CV, CH

Cerebellum
*4/*4, *1/*4, *1/*1

2D6 Liver
*1/*4, *1/*1, *4/*4
CYP2D6 genetic fast metabolizers differ from slow metabolizers

Bertilsson et al., 1989; Llerena et al., 1989; Llerana et al., 1993; Roberts et al., 2004; Gan et al., 2004

Other distinguishing features:
- Portion of Type A vs B
- Psychasthenia
- Inhibition of Aggression
- Novelty seeking
- Harm avoidance
- Fear of Uncertainty
- Shyness
Healthy individuals (N=188): CBP may reflect an ongoing biological process regulating the reactivity of the individual to emotional stimuli and the detection of signals evoking fear.
CYP enzymes in the Brain:
Understanding variation in drug and toxin response

Drug Activation
- Codeine and CYP2D
  - Analgesia and Abuse Liability

Drug Inactivation
- Propofol and CYP2B
  - Sedation
- Nicotine and CYP2B
  - Self-Administration

Toxin Inactivation
- MPP+ and CYP2D
  - Parkinson’s Disease
- Haloperidol and CYP2D
  - Tardiff dyskinesia

Toxin Activation
- Chlorpyrifos and CYP2B
  - Pesticide Neurotoxicity
Codeine is activated to Morphine by CYP2D6

- Morphine (MOR) has greater affinity for $\mu$-opioid receptors
- COD analgesia comes from conversion to MOR
- CYP2D6 poor metabolizers: no analgesia
- Pharmacological Inhibitors: decrease analgesia

Adler et al., 1955; Pert et al., 1973; Chen et al., 1991; Sindrup et al., 1991; Sindrup et al., 1992
Codeine enters brain faster than morphine

- Codeine is metabolized mainly by the liver, morphine then crosses into the brain

- Morphine less lipophilic than codeine, less permeable across BBB
- Brain uptake of morphine slower than codeine
- Morphine actively transported out of brain

Oldendorf et al., 1972; Bouw et al., 2000
CYP activity in the brain: Mechanism-Based Inhibitors (suicide inhibitors)

Substrate (Mechanism based inhibitor)

Metabolite, covalently bound to the enzyme
**Inhibition of brain CYP2D**

**Propranolol (PRL):** mechanism-based inhibitor of CYP2D

- Reactive metabolite binds covalently to enzyme
- Irreversible loss of enzyme function, requiring synthesis of new enzyme before activity is restored

Zhou et al., 2013

Propranolol

ACSF

COD

MOR

Analgesia

Masubuchi et al., 1994
Tail-flick test: rat model of nociception

- Tail-flick latency, TFL: time from start of heat exposure to withdrawal of tail
- Lengthening of TFL is interpreted as analgesia

Le Bars et al., 2001
CYP2D6 and Codeine

Morphine (10-15%)

CYP2D6 activates Codeine
- Morphine has greater affinity for µ-OR
- CYP2D6 poor metabolizers: no analgesia
- CYP2D6 Inhibitors: less analgesia

CYP2D6 and Codeine

Morphine (10-15%)

P-glycoprotein

BBB

Brain CYP2D

Hepatic CYP2D

OH

O

CH₃

Codeine

Codeine

Morphine

µ-OR

CYP2D6 and Codeine

Primary Objective
Examine the role of rat brain CYP2D in the metabolic activation and resulting analgesia of peripherally administered codeine

Study Design

Washout (One Week)

Baseline

Opioid Drug Administration

Analgesia Testing

Sacrifice

Wistar

Analgesia
- Baseline Tail-Flick Latency

Pretreatment
- Propranolol (Inhibitor)
  (20 µg in 4 µl cyclodextrin)
- Nicotine (Inducer)
  (7 x 1 mg/kg in saline)

Drug Levels
- Plasma
- Saphenous Vein
- Whole Brain
- Sacrifices

0 15 30 60
Time (Minutes)

Codeine
(20 mg/kg in saline)

Morphine
(3.5 mg/kg in dH2O)

Analgesia
- Tail-Flick Reflex

IP

ICV

SC
Inhibition of brain CYP2D

(Codeine tested 24 hr after propranolol)

Zhou et al. 2013
Inhibition of brain CYP2D

(Codeine tested 24 hr after propranolol)

Morphine

BBB

Hepatic CYP2D

Morphine

mu-OR

Zhou et al. 2013
Inhibition of brain CYP2D

(Codeine tested 24 hr after propranolol)

Zhou et al. 2013
Propranolol inhibition of brain CYP2D decreases codeine-induced analgesia.
Codeine, but not morphine, levels at 15 min after a codeine injection correlate between peripheral (plasma) and central (brain) compartments.
Analgesia correlated with brain, not plasma, morphine levels at 15 min after codeine-injection.
Propranolol pretreatment did not effect morphine-induced analgesia.

Between animal (n=6/group)
CYP2D6 levels are higher in some brain regions of smokers

No difference in hepatic CYP2D levels between smokers and non-smokers

Mann et al. 2008
Nicotine induces CYP2D6 in Monkeys
(Nicotine 0.3 mg/kg s.c. bid, 21 days)

Male African Green Monkey
(Cercopithecus aethiops)

Frontal Cortex Western Blot

Saline (N=6)  Nicotine (N=6)

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Saline</th>
<th>Nicotine</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Frontal cortex</td>
<td>1</td>
<td>X 2.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>X 1.7</td>
<td>0.001</td>
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<tr>
<td>Putamen</td>
<td>X 1.6</td>
<td>0.006</td>
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<tr>
<td>Cerebellum</td>
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<tr>
<td>Frontal cortex</td>
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Induction of brain CYP2D

(Codeine tested 8 hr after chronic nicotine)
Induction of brain CYP2D

(Codeine tested 8 hr after chronic nicotine)

Yue et al. 2008
Induction of brain CYP2D

(Codeine tested 8 hr after chronic nicotine)

Tail Flick Latency (s, mean ± SEM)

- Day 1
- Day 7

Pre Post (8 h)

Brain CYP2D

Codeine

Morphine

mu-OR

Yue et al. 2008
Induction of brain CYP2D

(Codeine tested 8 hr after chronic nicotine)

![Graph showing induction of brain CYP2D](image)

- **Tail Flick Latency (s, mean ± SEM)**
  - Day 1
  - Day 7

- **Plasma level (ng/ml, mean ± SEM)**
  - Time after nicotine injection (h)

Yue et al. 2008
Nicotine induction of brain CYP2D increases **codeine**-induced analgesia

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**Analgesia (%MPE, mean ± SEM) vs. Time after codeine injection (min)**

- **Vehicle**
- **Propranolol (Inhibitor)**

**AUC (fold change vs. vehicle)**

- **0-30 min**
- **15 min**

**Plasma Morphine**

- Within animal (n=12)
Nicotine induction of brain CYP2D increases codeine-induced analgesia.
Nicotine pretreatment did not effect morphine-induced analgesia

Within animal (n=16)
Nicotine induction of brain CYP2D increases codeine-induced analgesia
Effect of nicotine (brain CYP2D inducer) on codeine-induced analgesia was reversible.
Brain CYP2D contributes to **codeine**-induced analgesia at early time points

- Brain CYP2D plays a significant role in the metabolism and initial analgesia from peripheral codeine
  - Despite first-pass metabolism (intraperitoneal administration)

- Inhibiting and/or inducing brain CYP2D did not effect:
  - Baseline tail-flick latencies (nociception)
  - Plasma morphine levels after codeine-injection
  - Morphine-induced analgesia (antinociception)
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