# Monocarboxylate Transporters in Drug Disposition: Role in the Toxicokinetics and Toxicodynamics of the Drug of Abuse GHB

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# Monocarboxylate Transporters (MCT)

- Two families of transport proteins with substrates consisting of important metabolic products such as lactate, pyruvate and ketone bodies, as well as monocarboxylate drugs.

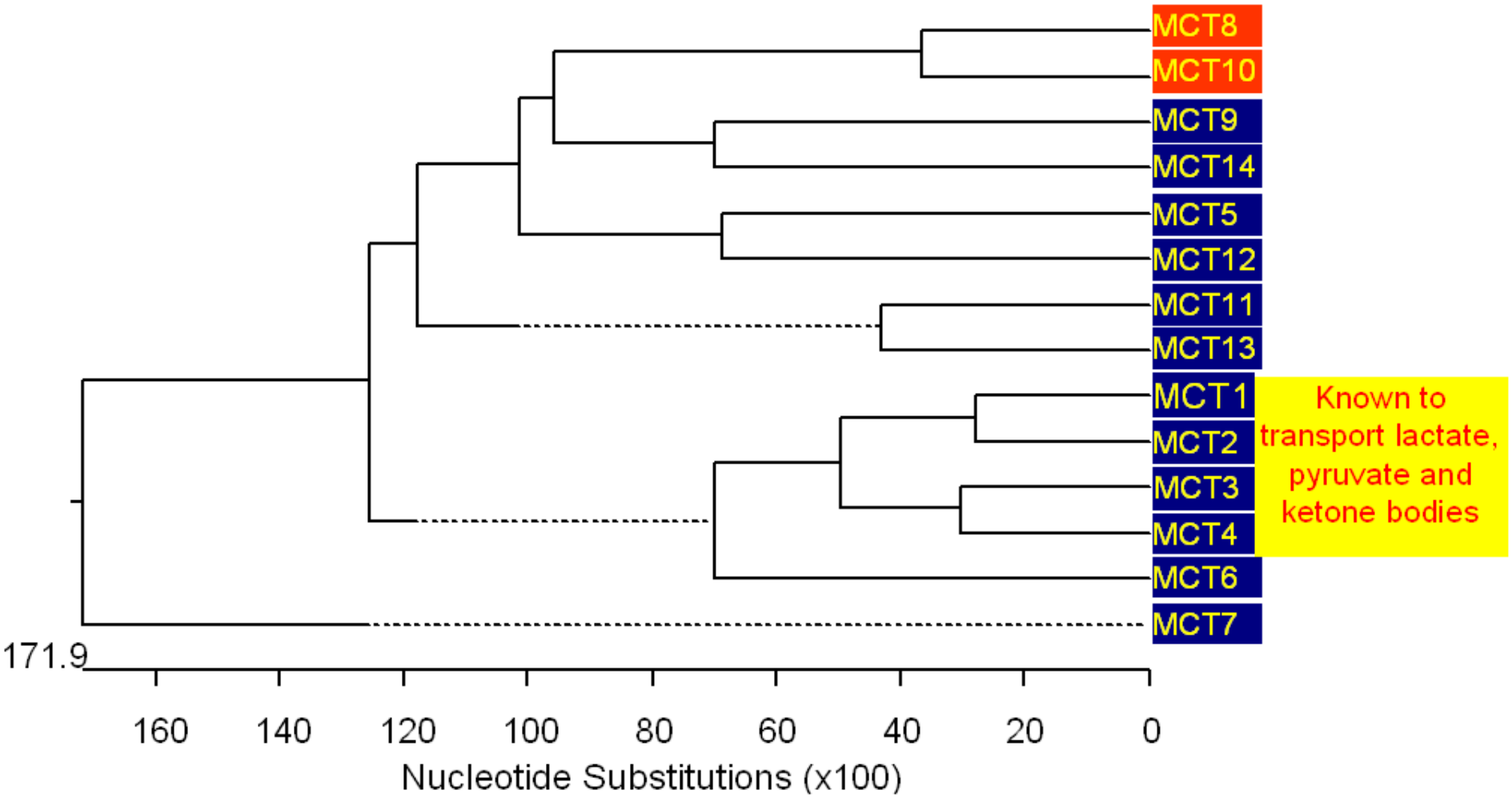
  SLC16- proton-coupled

  SLC5 – sodium-dependent

# Relatedness of human MCT isoforms

The natural substrates for MCT5, MCT6 , MCT7, MCT9 and MCT11-MCT14 are unknown but probably do not include lactate and pyruvate

MCT8 is a thyroid hormone transporter and MCT10 (TAT1) is a neutral amino acid transporter

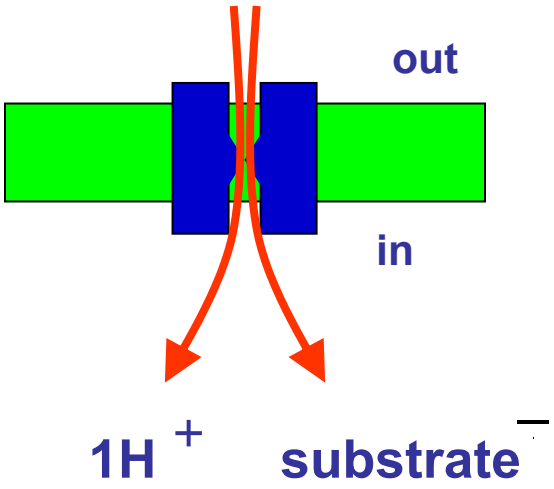



Andrew Halestrap, Department of Biochemistry , University of Bristol

# MCT 1-4

- Proton-linked transport of monocarboxylates such as lactate, pyruvate and ketone bodies across membranes plays a critical role in the metabolism and pH regulation of most cells.

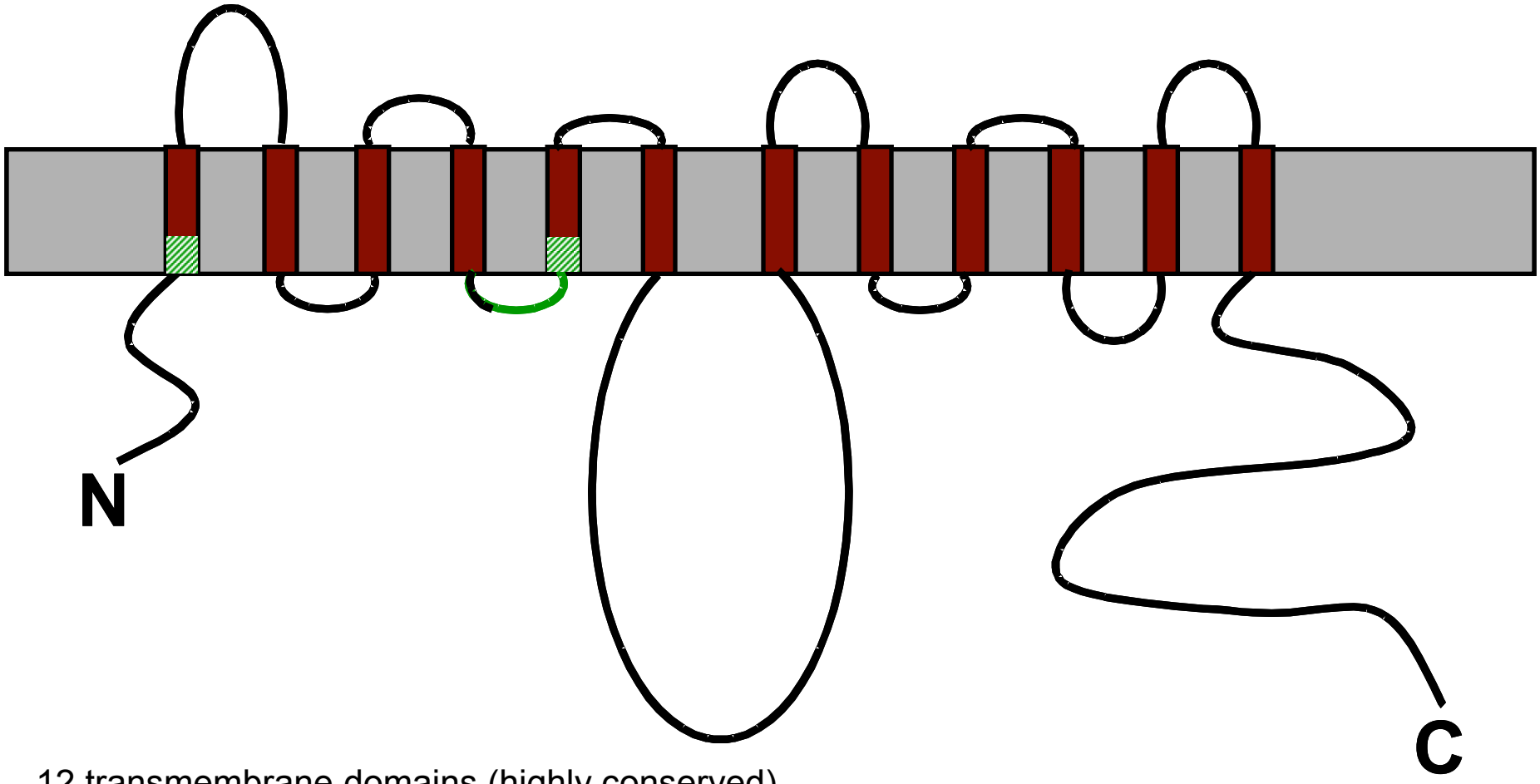
SLC16A1- MCT1
SLC16A7-MCT2
SLC16A3-MCT4
SLC16A8-MCT3




MCT1-4 $H^+$-coupled

# Topology of MCT1

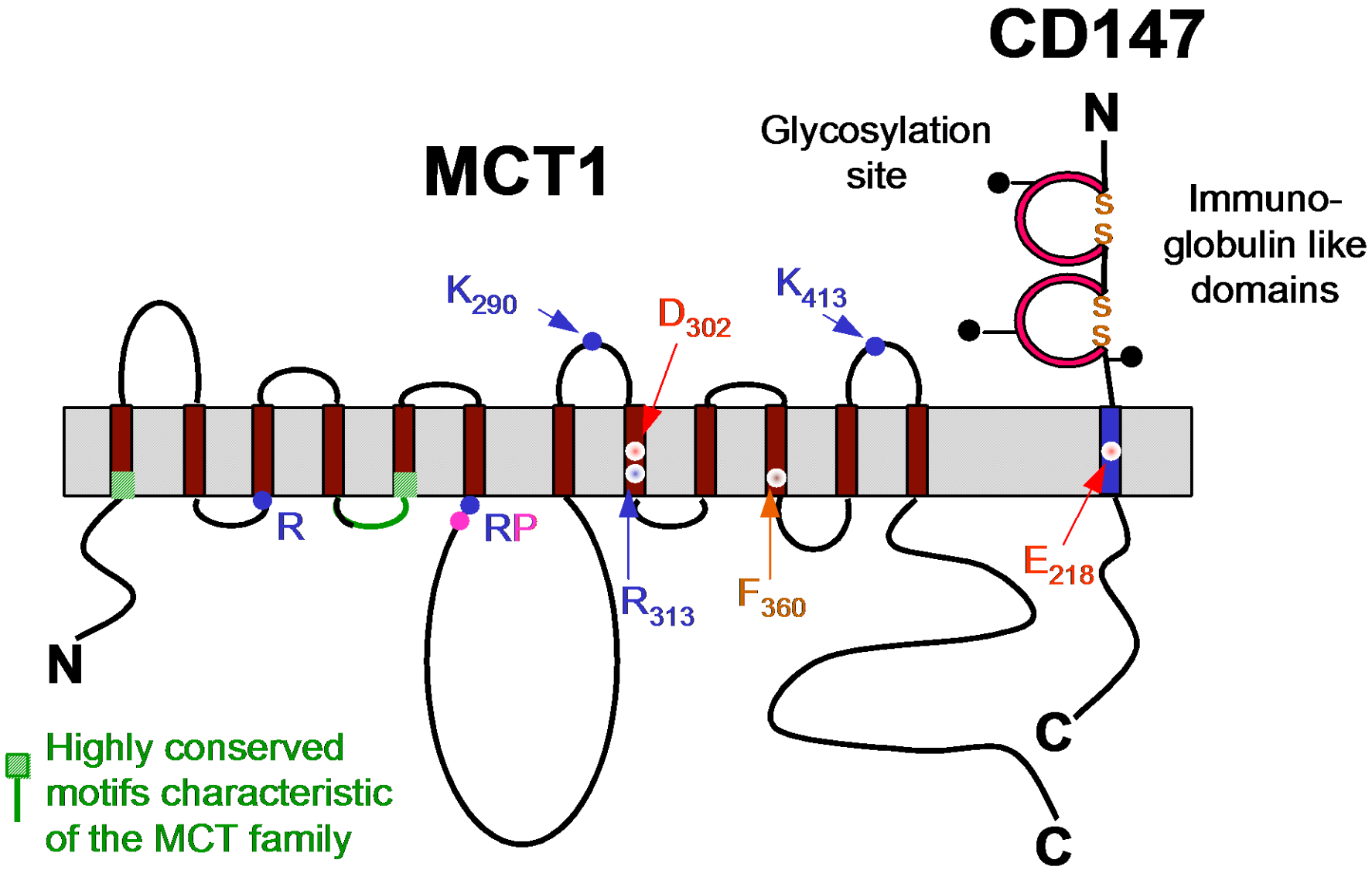
Confirmed by selective proteolysis and labelling of red blood cells




-12 transmembrane domains (highly conserved)
- Intracellular N- and C- termini
- A large intracellular loop between domains 6 and 7

http://www.bristol.ac.uk/biochemistry/halestrap/mct.html

# The lactate carrier is closely associated with another membrane protein of the immunoglobulin superfamily



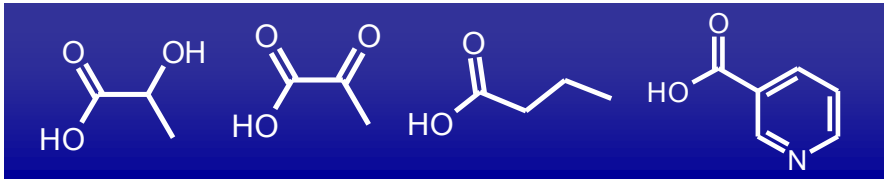
# MCTs 1, 2 &4 function in conjunction with an ancillary protein

- Basigin (CD147), a glycoprotein that belongs to small subset of the immunoglobulin superfamily, is necessary for the function of MCT1 and MCT4; it is the target for organomercurial inhibition of these MCTs.
- The ancillary protein for MCT2 is Embigin (gp70).
- Interaction is necessary for membrane trafficking, and the direct protein-protein interaction in the membrane is necessary for activity.

# Substrates of MCT1-4

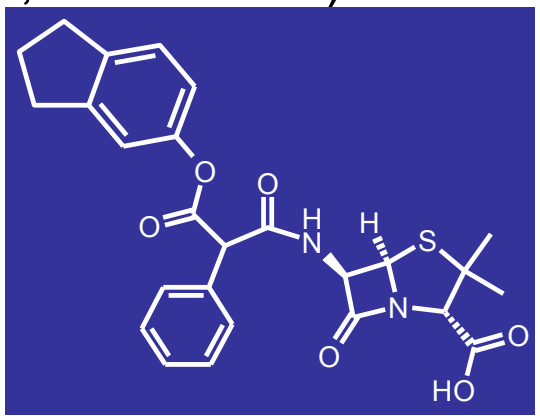
## Natural Substrates

- A variety of physiologically relevant substrates (e.g. lactate, pyruvate, butyrate)



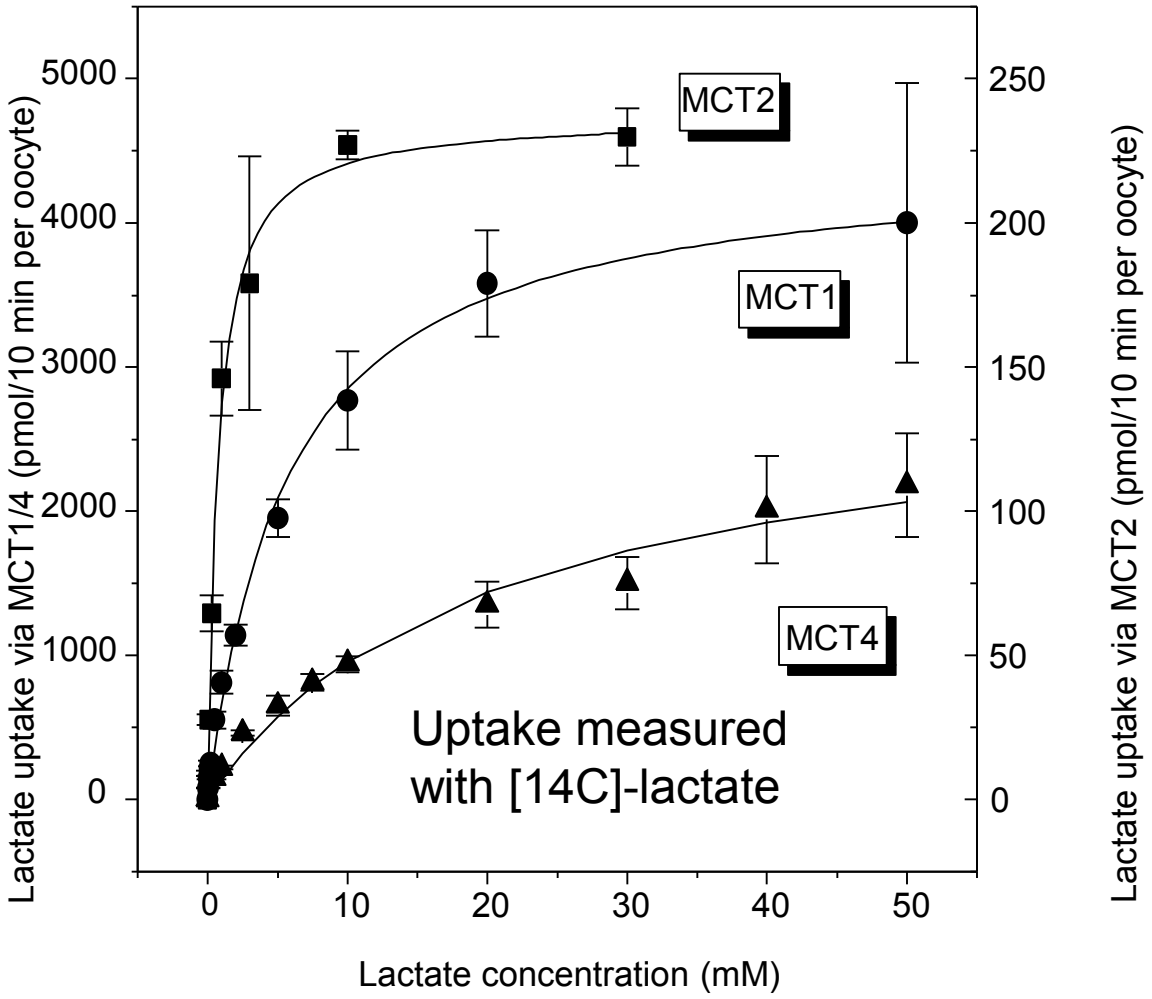

## Clinically relevant drugs

- NSAIDs (salicylic acid, ibuprofen)
- Beta-lactam antibiotics (phenethicillin, propicillin, carindacillin)
- HMG-CoA reductase inhibitors (simvastatin, atorvastatin)
- Valproic acid
- XP13512 (gabapentin prodrug)
- 6-mercaptopurine
- Quercetin, caffeic acid




Carindacillin

# Kinetics of L-lactate transport into Xenopus oocytes transfected with MCT1, MCT2 and MCT4




Stefan Bröer, Angelika Bröer, Hans-Peter Schneider, Roger Dallwig and Joachim W. Deitmer, Tübingen

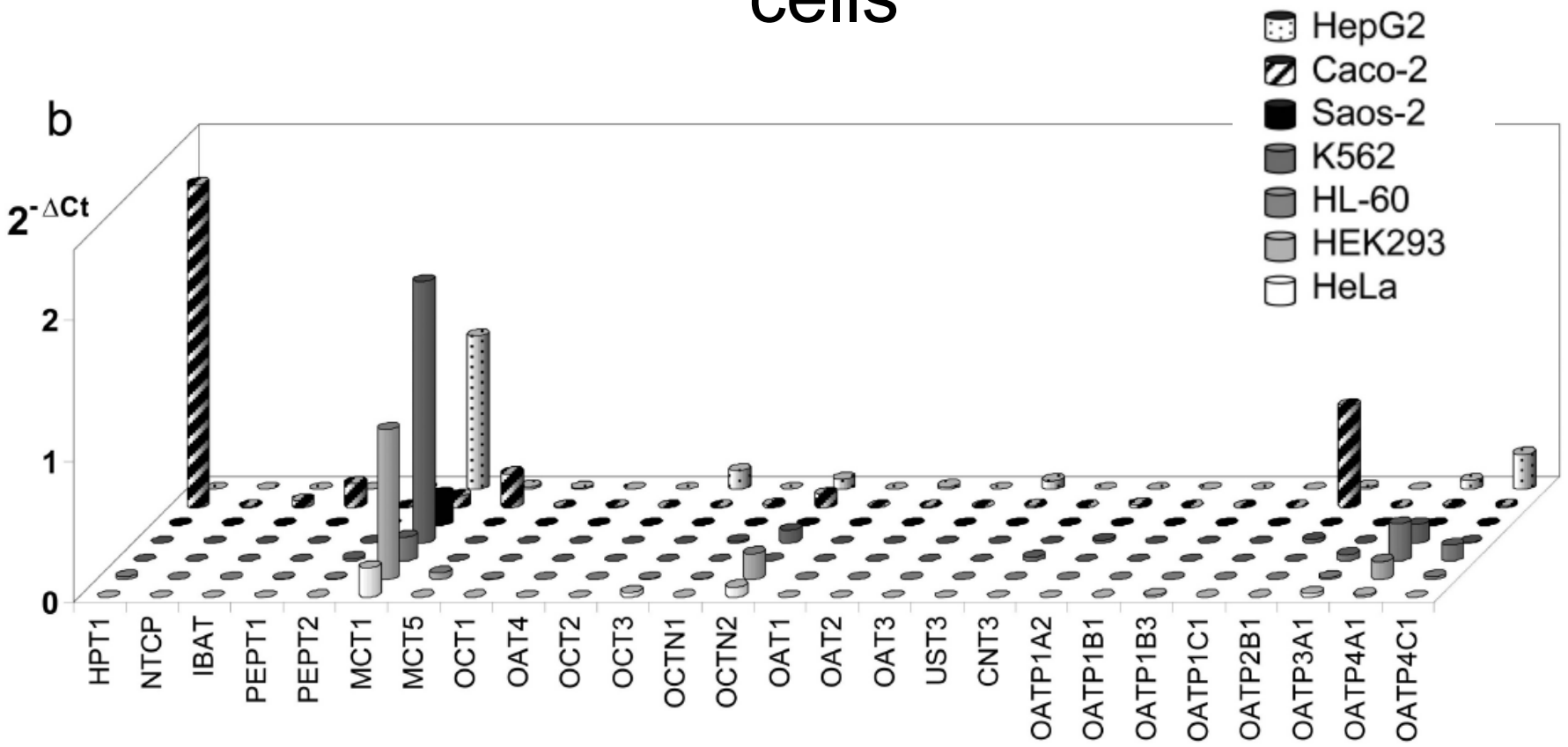
# Tissue Distribution of MCTs

- MCT1: expressed ubiquitously in almost every tissue and serves as the carrier for lactate flux across the plasma membrane of most cells

MCT2-4: more tissue-specific role

- MCT2: major MCT in rodent neurons, but in human brain its expression is minimal; liver expression
- MCT3: expressed in the retinal pigment epithelium; located at the basolateral membrane, where it functions in tandem with MCT1 on the apical side
- MCT4: expression is limited to highly glycolytic tissues (skeletal muscle, heart and liver); works synergistically with MCT1

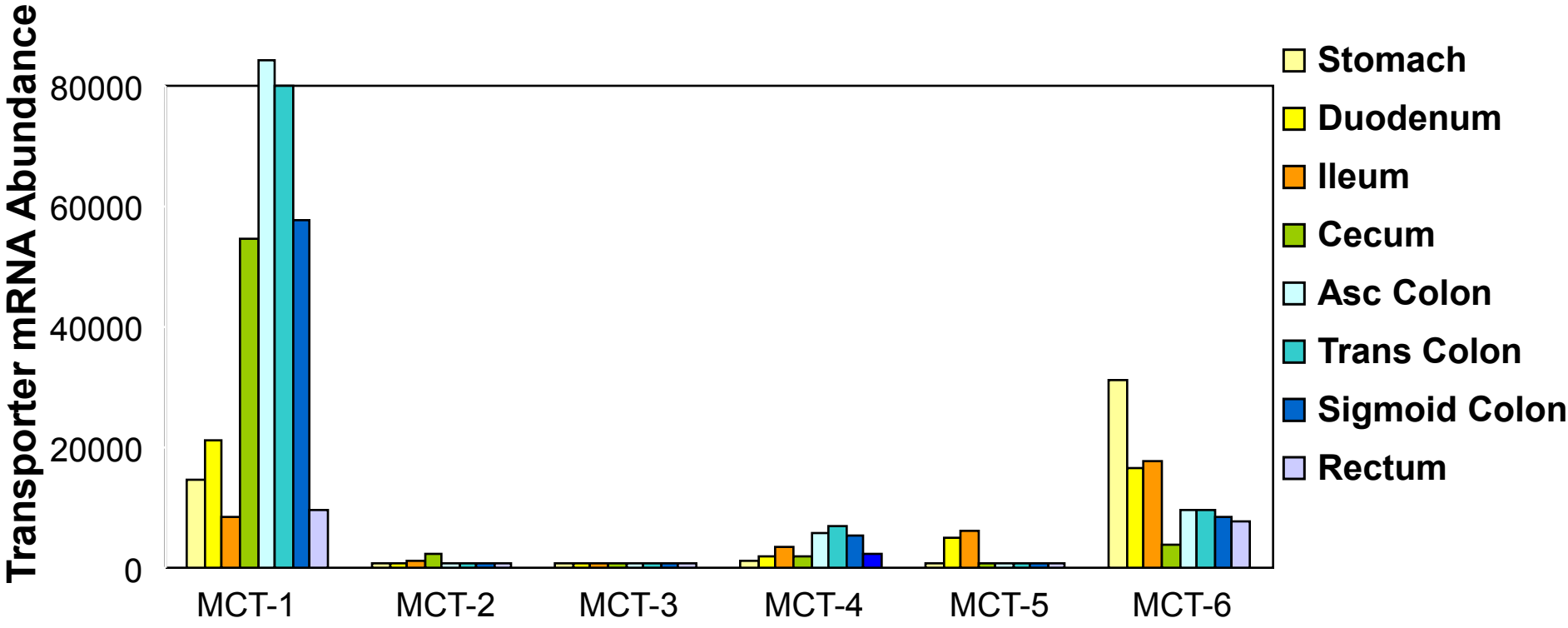
# Relative gene expression in HeLa, HEK293, HL-60, K562, Saos-2, Caco-2 and HepG2 cells




Plus excellent correlation of mRNA and protein expression

Ahlin, Artursson Drug Metab Disp, 2009

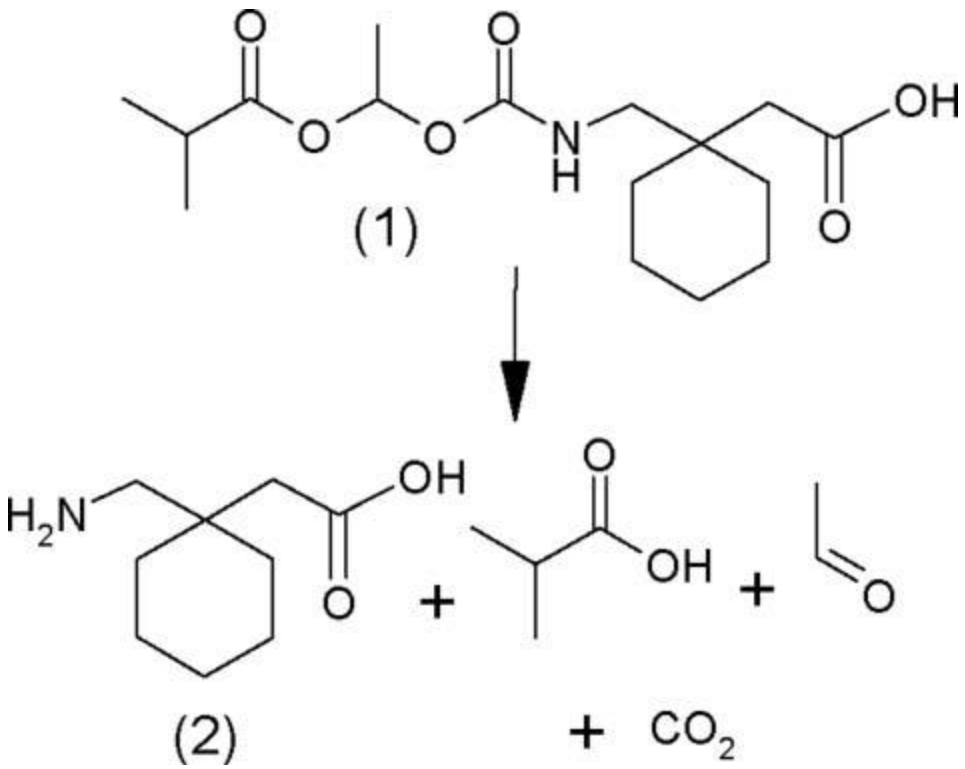
# MCT Transporters in the Human Intestine




$\Rightarrow$ MCT-1: Highly expressed in all segments of the small intestine and colon

Cundy KC, Xenoport, March 8, 2005

# Prodrug of Gabapentin (XP13512, Solzira™ )

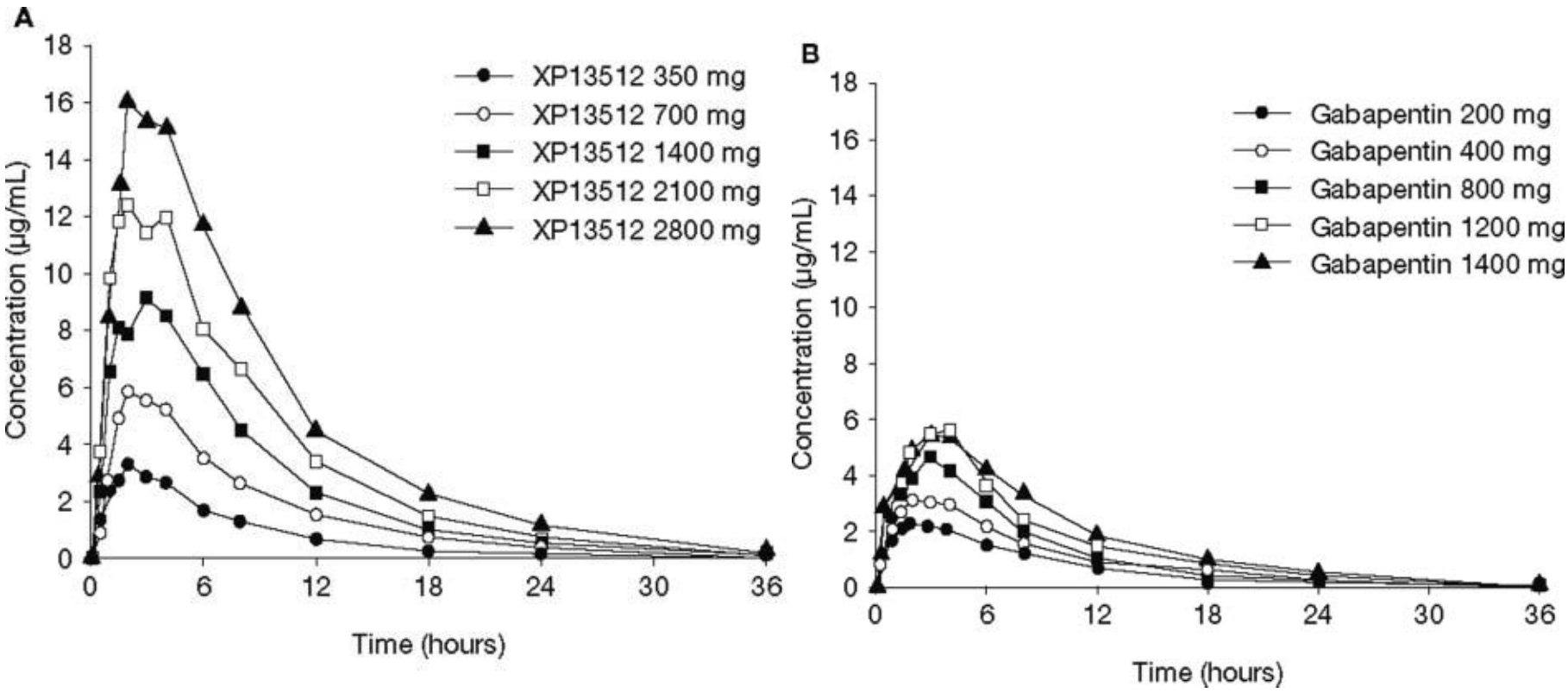



XP13512 undergoes enzymatic hydrolysis *in vivo* to liberate gabapentin, isobutyrate, acetaldehyde, and $CO_2$

XenoPort Inc.

# XP13512 (prodrug) vs. Neurontin: Concentrations of Gabapentin in Humans

*Mean concentrations of gabapentin in blood after oral dosing of approximately equimolar doses of (A) XP13512 capsules (n = 8 per dose level) or (B) oral gabapentin (n = 10 per dose level).*




**Cundy et al., J Clin Pharmacol 2008;48:1378-1388**

# Sodium-coupled monocarboxylate transporter (SMCT)

SMCT1 (SLC5A8, high affinity)

SMCT2 (SLC5A12, low affinity )

**SMCT1:**

- **Expression:**

  kidney (apical, tubular epithelial cells ), GI (apical, colonic and intestinal epithelial cells), thyroid gland, brain, salivary gland, retina

- **Substrates**:

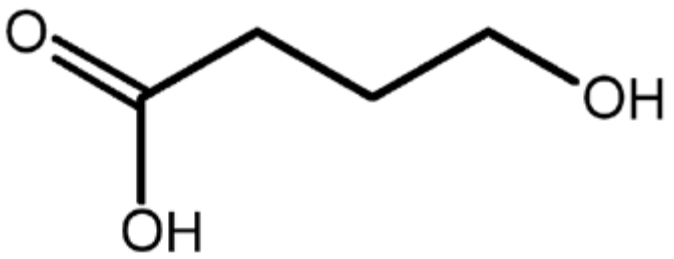
  short chain fatty acids (butyrate, lactate, acetate, propionate, pyruvate), medium-chain fatty acids (hexanoate, pentanoate, heptanoate, octanoate), ketone bodies (β-hydroxybutyrate and acetoacetate), nicotinate, salicylate, and 5-aminosalicylate

# γ-Hydroxybutyric Acid:

# MCT and SMCT substrate

# Gamma-hydroxybutyric acid (GHB)

- **Naturally occurring short-chain fatty acid formed from GABA**
  - MW 104
  - pKa 4.7
  - Highly hydrophilic
  - Negligible protein binding




- **Clinical uses:**
  - Narcolepsy (USA)
  - Alcohol withdrawal (Europe)

- **Marketed as Xyrem®**

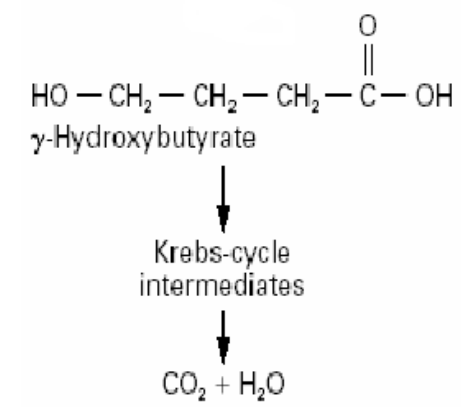
www.erowid.org

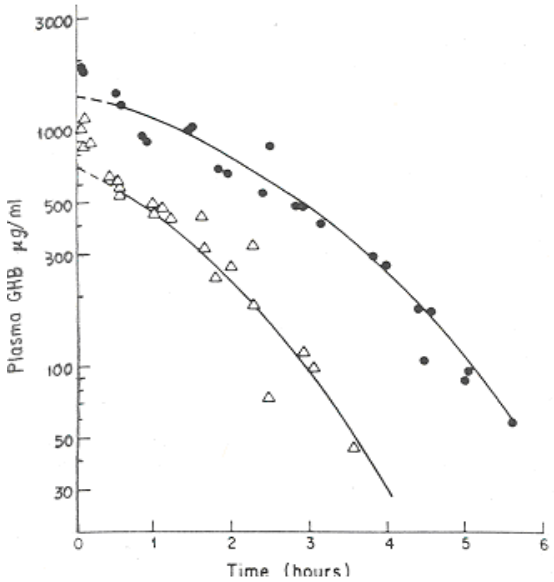
# GHB Toxicity and Abuse Potential

- **Wide spread abuse of GHB (Liquid Ecstasy) and its precursors (1,4-butanediol and gamma-butyrolactone):**
  - Body building (growth hormone releasing effects)
  - Recreational use for euphoric effects (club drug)
  - Drug-facilitated sexual assault
- **Typical recreational dose: 1 – 3 grams typically in liquid form**
- **GHB overdoses have resulted in 1084 ER visits in 2006 (DAWN, NIDA)**
  - Likely an underestimate due to detection difficulties and lack of reporting
  - Second most common drug detected in urine of young people presenting with drug-induced coma, just behind cocaine
- **Adverse effects characterized by CNS depression and cardiovascular effects**:
  - Respiratory depression, seizures, nausea, vomiting, unconsciousness, coma and death
- **Current treatment strategies involve supportive care**

# Pharmacokinetics of GHB

- GHB is eliminated by metabolism to $CO_2$
- Nonlinear PK of GHB has been demonstrated in rats (*Lettieri and Fung, 1978*) and humans (*Palatini, et al. 1993*)
- Urine concentrations are low; only 3-6% of GHB total dose
- Urine concentrations of GHB are very high in GHB overdosed patients [*Sporer et al. 2003*]




*Lettieri JT and Fung HL JPET 1978*

**The goal of this research is to identify specific therapeutic interventions for the treatment of GHB overdoses.**

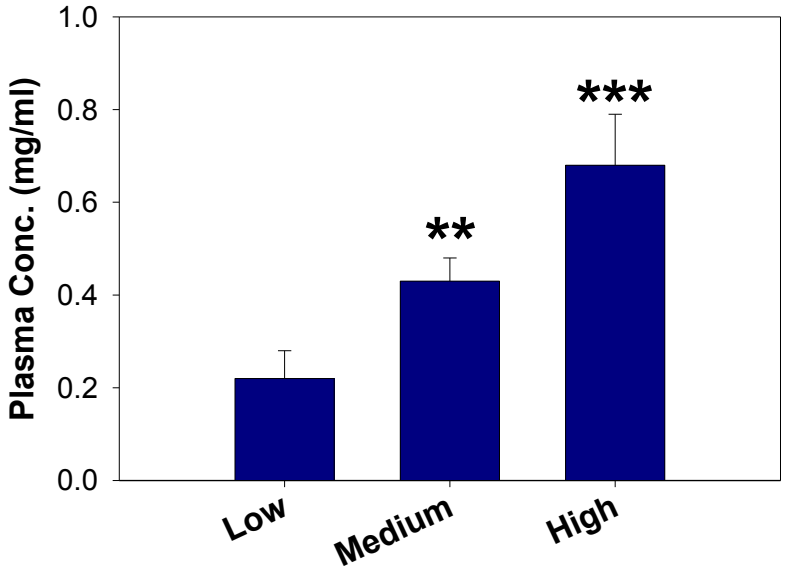
# Toxicokinetics of GHB

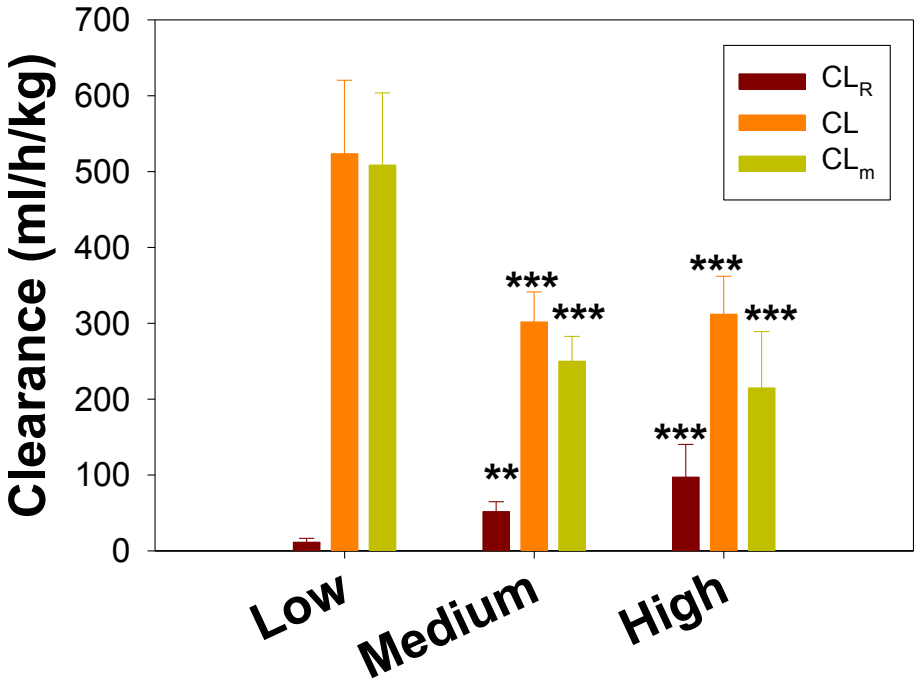
Urine concentrations are low (3-6% of dose) after low doses but much higher after overdoses.

Hypothesis:

GHB is actively reabsorbed by the kidney by MCTs resulting in nonlinear renal clearance

# Non-linear Renal Clearance




$$CL_t = \frac{K_{0,GHB}}{C_{ss,GHB}}$$

$$CL_R = \frac{A_{e,urine}/t}{C_{ss,GHB}}$$

$$CL_m = CL_t - CL_R$$

** P< 0.01, *** P<0.001

Morris et al, J Pharmacol Exp Ther, 2005

# GHB Renal Clearance

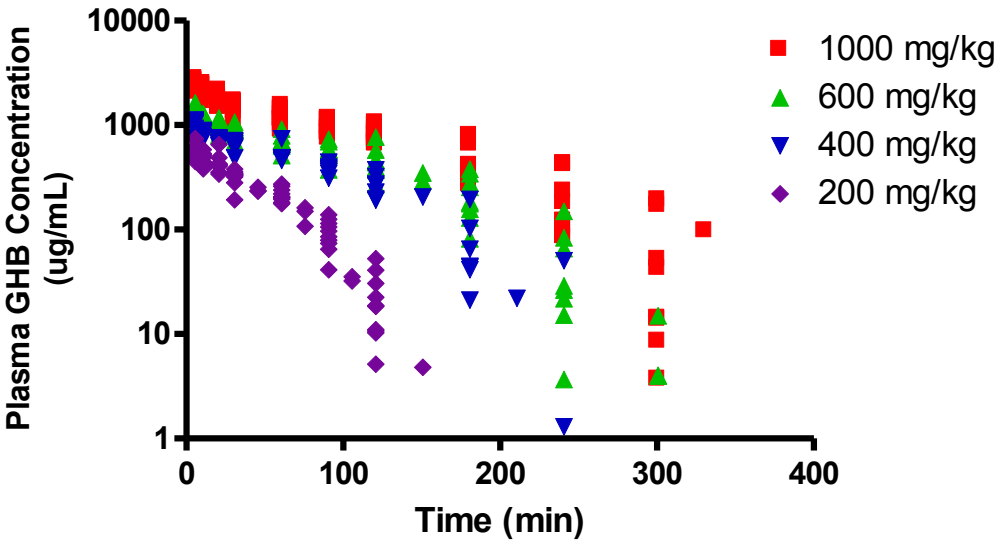
| Dose (mg/kg/h) | Cp (mg/ml) | CLr (ml/kg/h) | CLt (ml/kg/h) | fe(%) |
|---|---|---|---|---|
| 108.3 (n=3) | 0.23±0.03 | 14.9±5.1 | 523±96 | 2.9± 1.0 |
| 127.0 (n=4) | 0.43±0.05 * | 51.8±13.0 * | 302±40 ** | 17.1±3.6 ** |
| 208.3 (n=4) | 0.67±0.12 *** | 97.1±43.1 *** | 312±50 * | 32.1±16.2 *** |

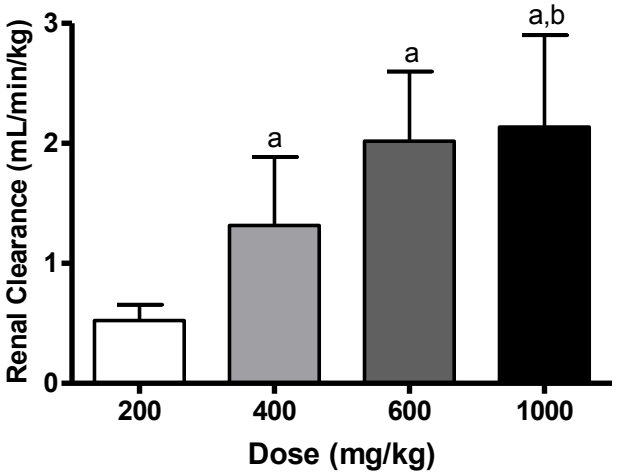
Compared to low dose

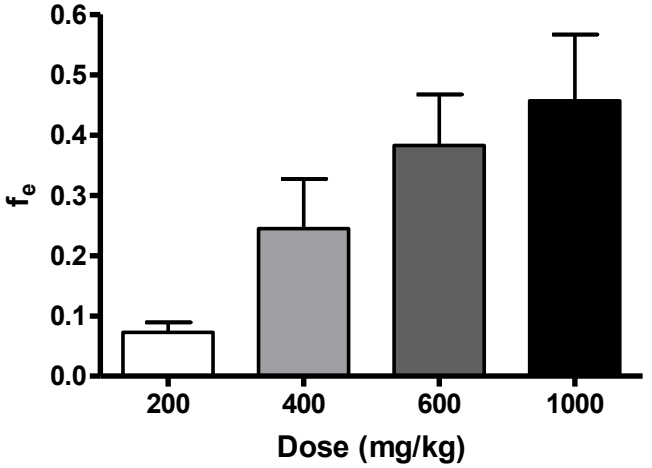
* p< 0.05; ** p<0.01; *** p<0.001

One-way ANOVA followed by Dunnett's test

# GHB Exhibits Nonlinear PK



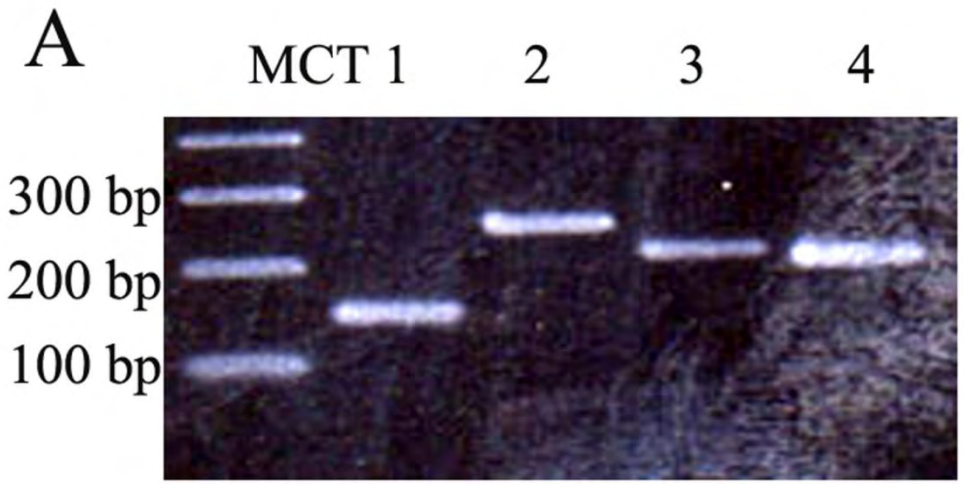




**Dose-dependent increases in $Cl_R$ suggest active renal reabsorption of GHB**

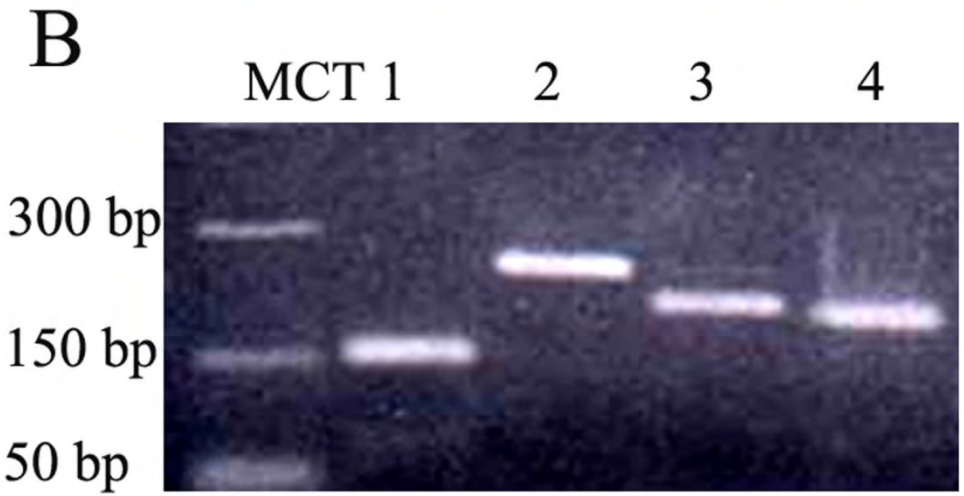
Mean ± SD. N = 7-9

# Expression of mRNA of MCTs in HK-2 cells and human kidney cortex

**Human Kidney**



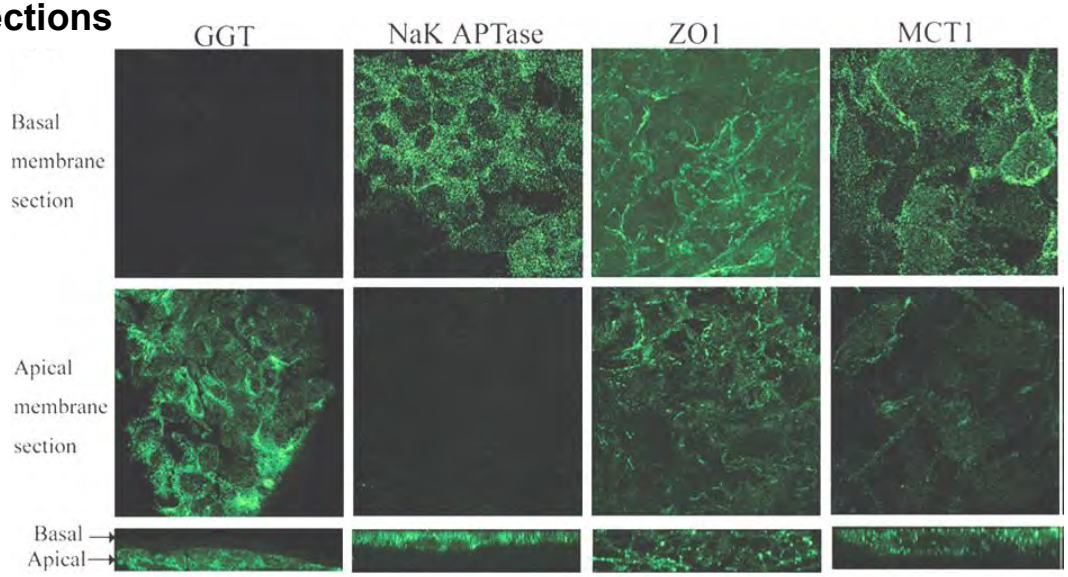

**HK-2 Cells**




Wang,Q et al., Mol Pharmaceutics, 2006

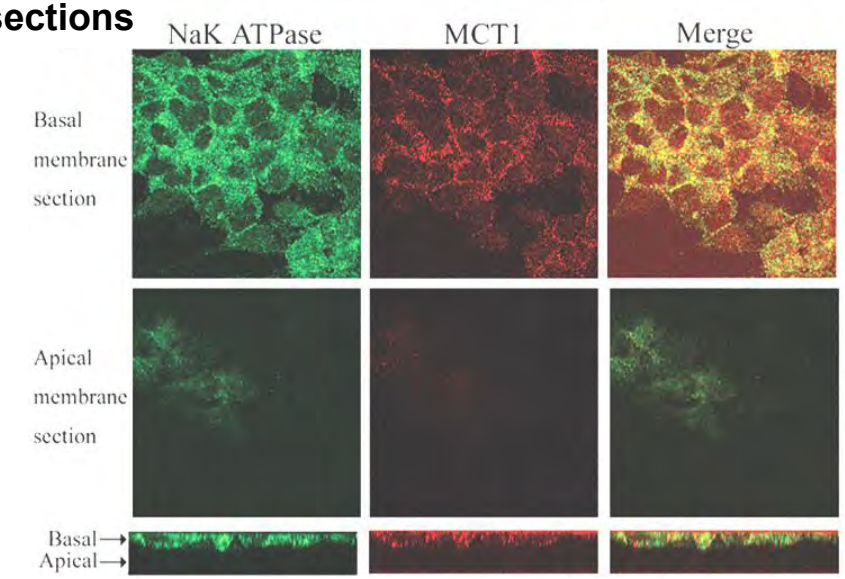
# MCT1 in human kidney HK-2 cells

**XY sections**




**MCT1 (FITC-conjugated antibody) is distributed predominantly on the basal membrane of HK-2 cells.**

**XZ sections**

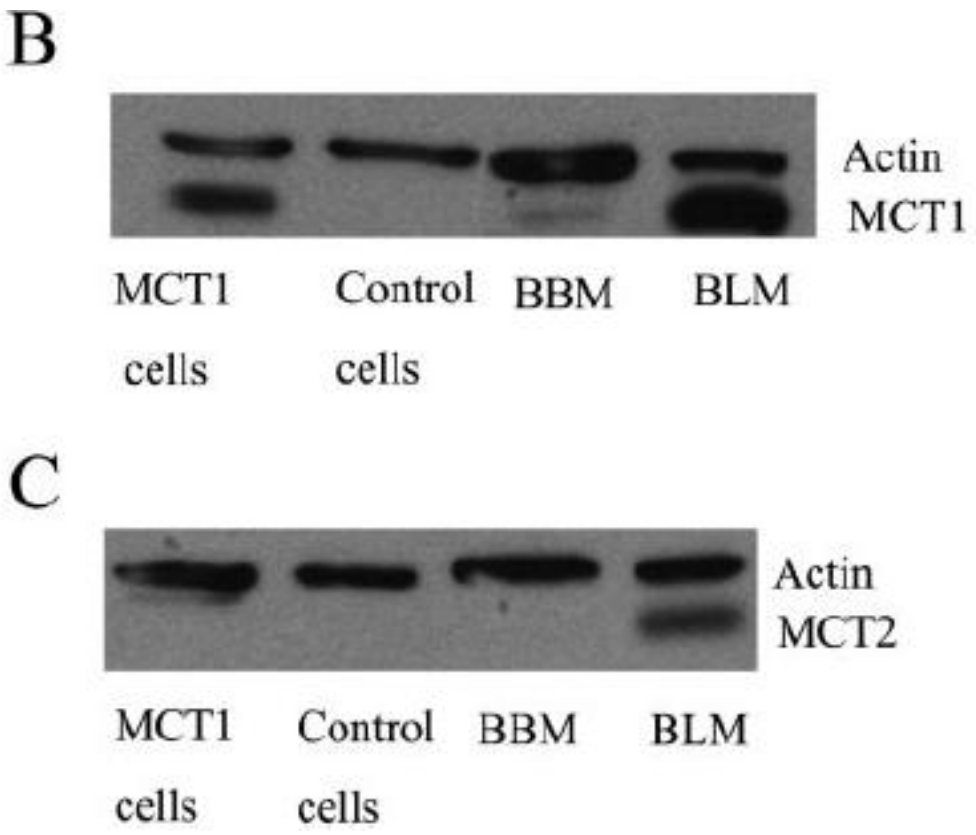



**The overlay of MCT1 (red, stained with rhodamine-conjugated antibody) and Na+/K+-ATPase (green). The two proteins are distributed on the same side of the cell membrane, and they are partially co-localized.**

Wang Q et al. (2006) *Molecular Pharmaceutics* 3(6):675-685

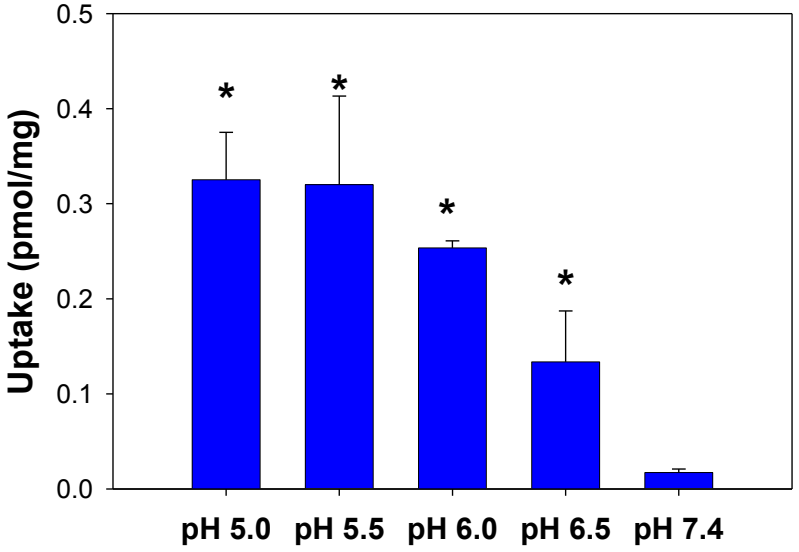
# Rat kidney expression of MCT1 and MCT2

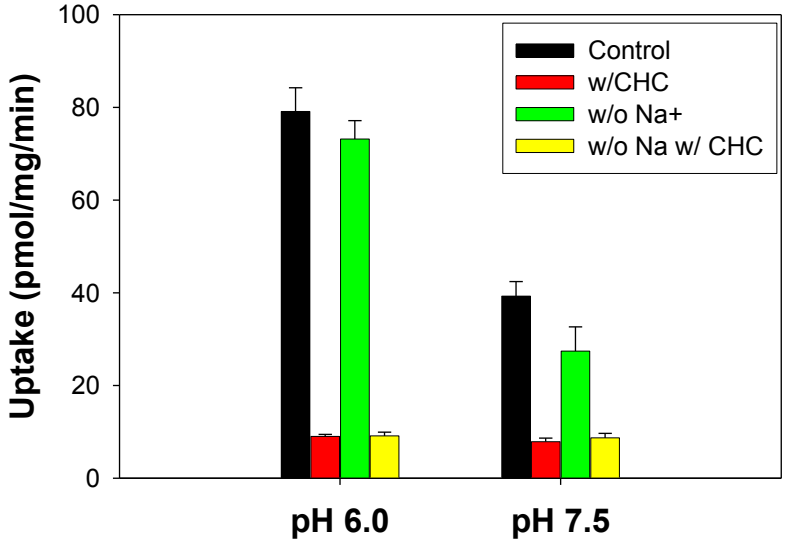
Western blot analysis




Wang et al., JPET 318:751-761, 2006

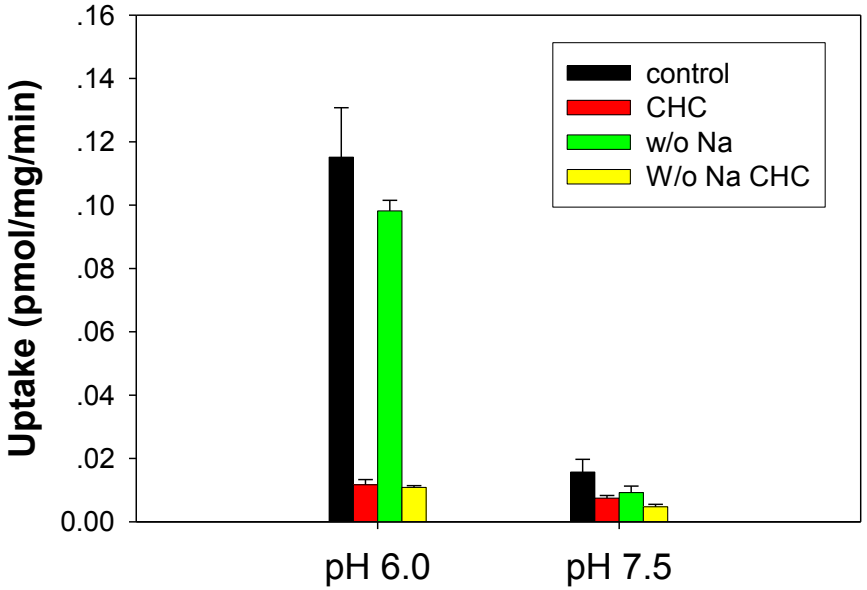
# pH and Sodium effects on L-lactate uptake by HK-2 cells



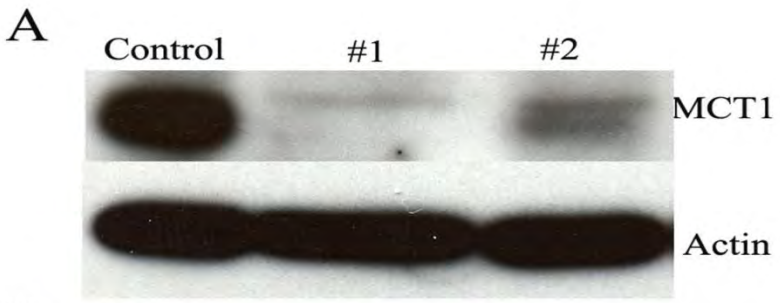



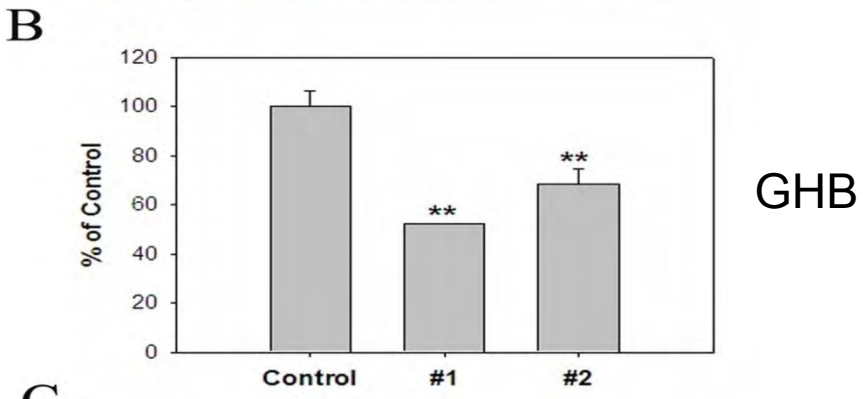

- Uptake of L-lactate decreased as pH increased
- Uptake of L-lactate was not affected by the presence or absence of sodium ion
- Uptake of L-lactate was inhibited by CHC (α-cyano-hydroxycinnamate 2mM), a MCT inhibitor

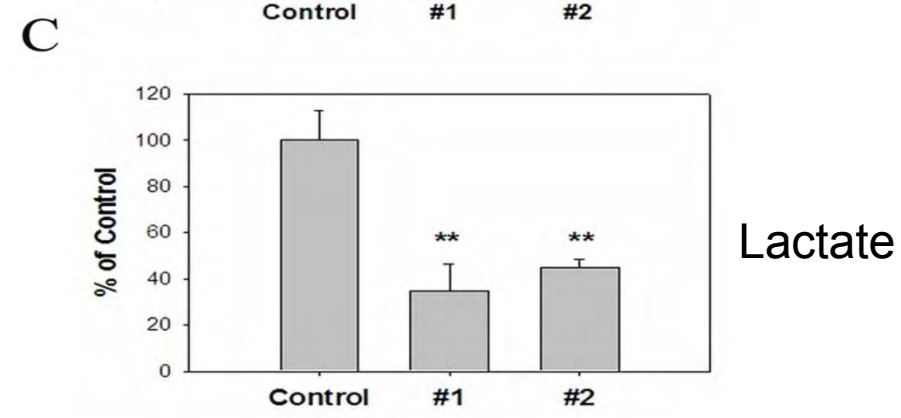
# GHB transport in HK-2 cells



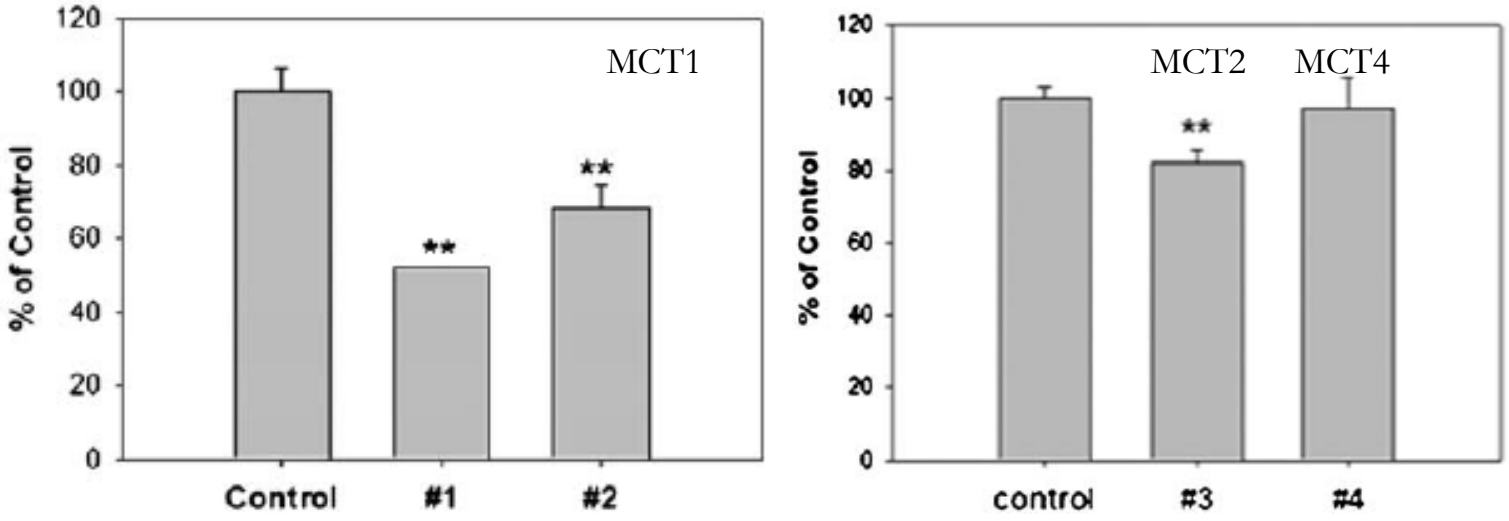

| Parameters | Mean±SD |
|---|---|
| $V_{max}$ (pmol/mg/min) | 27.6±9.3 |
| $K_m$ (mM) | 2.07±0.79 |
| P (μL/mg/min) | 0.54±0.15 |



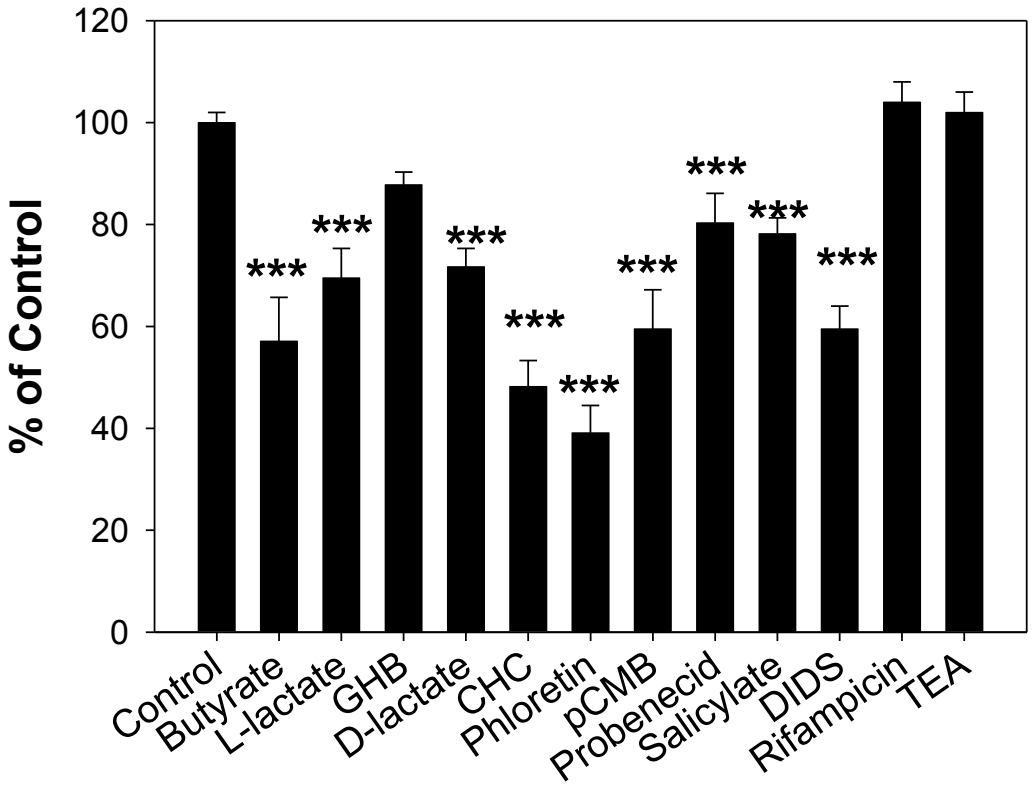
# MCTs Involved in GHB Uptake




- RNA interference studies using siRNA
- Significant decreases in MCT1 and MCT2 mediated GHB uptake in HK-2 cells
- MCT1 represents an important transporter for GHB uptake

Wang Q et al. (2007) Pharm Res

# Effects of Inhibitors on Uptake of GHB by HK-2 Cells




*** p< 0.001

TEA: Triethylamine (2mM);

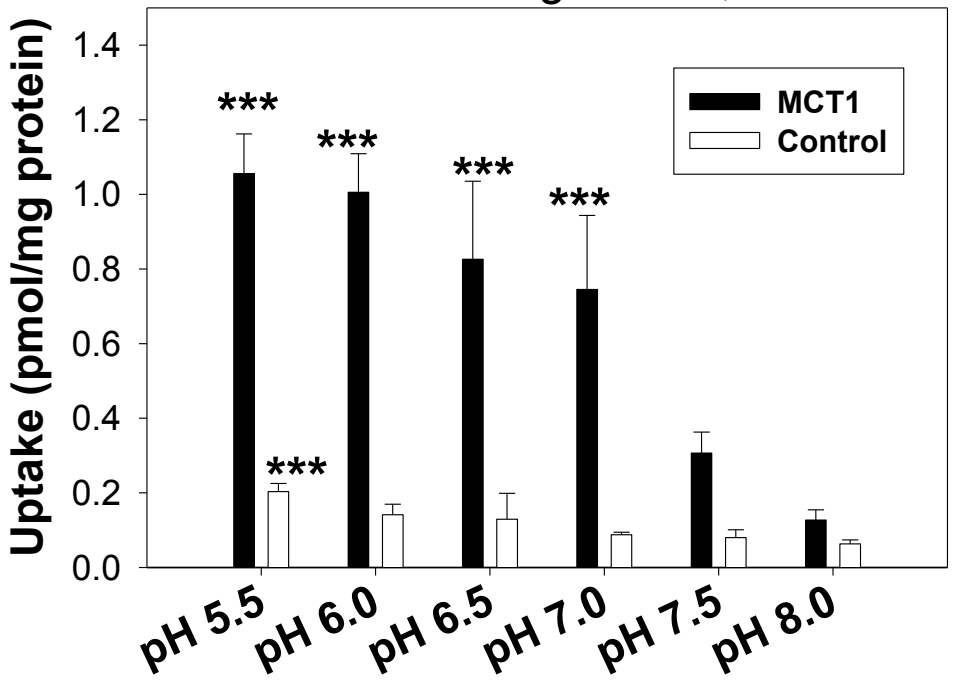
CHC: Alpha-cyano-4-hydroxycinnamate (2mM); pCMB= p=chloro-mercuribenzoic acid

Probenecid (0.1 mM), Salicylate (2mM), GHB (5mM), D, L-lactate (5mM)

# MDA-MB231/rMCT1 cells

pH-dependent uptake into MDA-MB231/rMCT1 cells

Wang Q et al, J Pharmacol Exp Ther, 2006




MDA-MB231 cells: human breast-cancer cells lines; lack of MCT1 expression

---*Garcia CK, et al, cells. 1994*

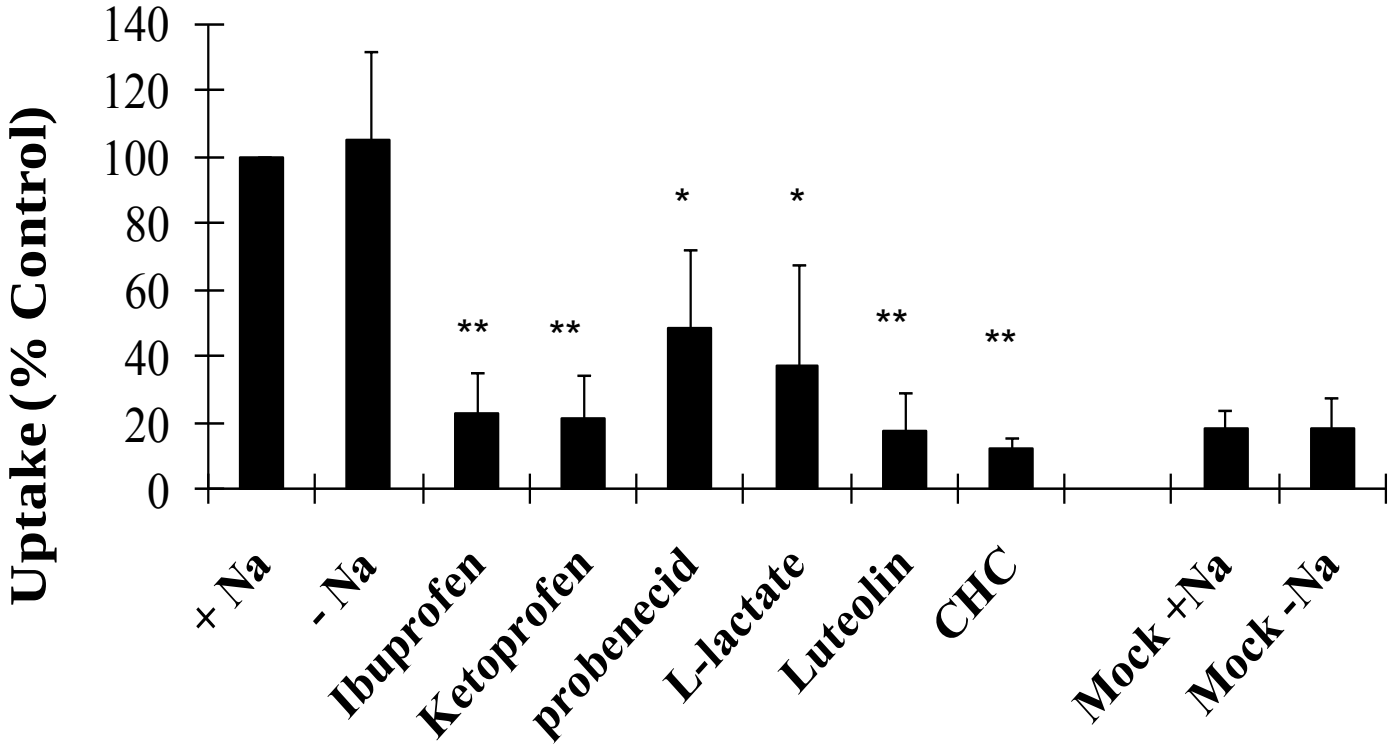
Rat MCT1 expression

(Prof Tamai and Tsuji, Kanazawa Univ, Japan)

---*Tamai I, et al, J. Pharm. Pharmacol. 1999*

| Parameters | MCT1 cells |
|---|---|
| $K_m$ (mM) | 4.6 ± 1.5 |
| $V_{max}$ (pmol/mg/s) | 1350 ± 613 |

# Effect of inhibitors on MCT1-mediated GHB uptake




1min uptake by **rat MCT1 gene-transfected MDA-MB231 cells** at RT
pH 6.0, GHB 5 μM, luteolin 0.05mM, all other inhibitors 1mM ,
* $p < 0.05$, ** $p < 0.01$

Cui and Morris, DMD 2009

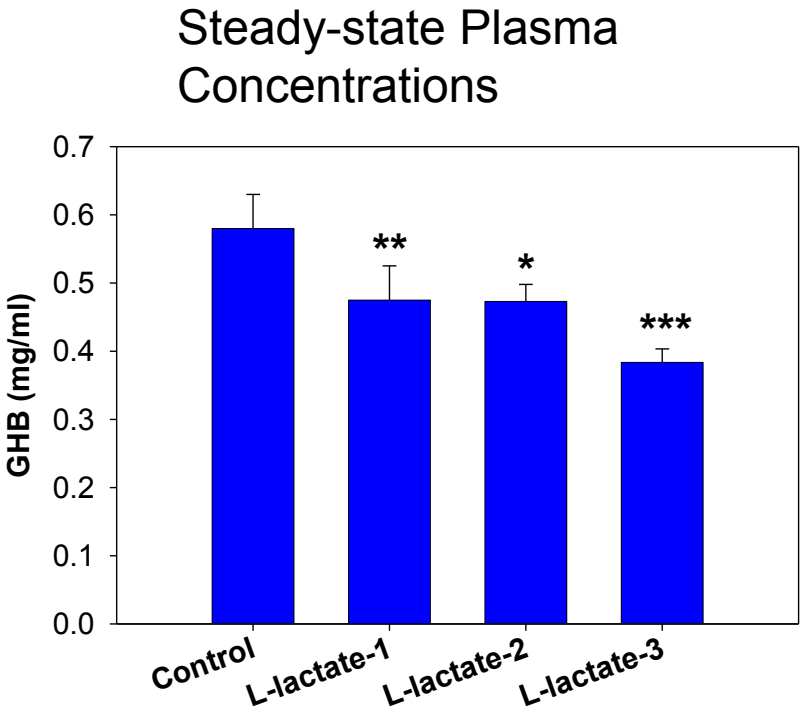
# Hypothesis

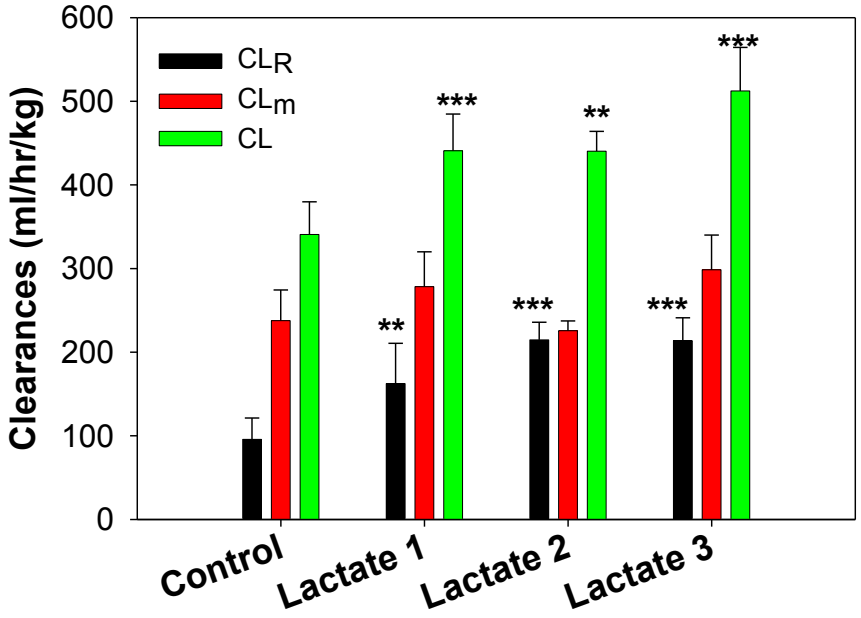
Aim: Determine effects of MCT inhibitors on the TK and TD of GHB

Hypothesis: Inhibiting MCTs represents a potential strategy of treating overdoses of GHB

- L-Lactate as a MCT Inhibitor

# Effects of L-lactate on GHB PK




Lactate 1: 60.5 mg/hr/kg

Lactate 2: 121 mg/hr/kg

Lactate 3: 302.5 mg/hr/kg

* p< 0.05; ** p<0.01; *** p<0.001

Wang et al, Drug Metab Disp 36:2244. 2008

# Effects of L-lactate on GHB TK

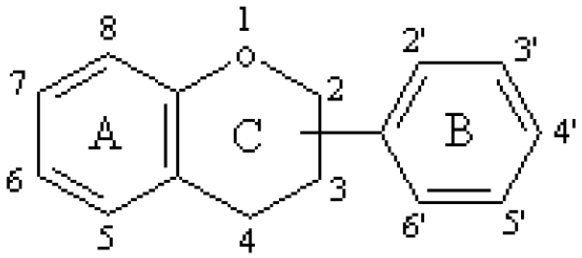
| | Control | L-lactate-1 | L-lactate-2 | L-lactate-3 |
|---|---|---|---|---|
| Plasma [GHB] (mg/ml) | 0.62 ± 0.11 | 0.48 ± 0.04** | 0.46 ±0.01** | 0.38 ± 0.02** |
| Total clearance (ml/hr/ kg) | 340 ± 39 | 441 ± 44*** | 440 ± 24** | 512 ± 52*** |
| Renal clearance (ml/hr/kg) | 96 ± 26 | 163 ± 48** | 214 ± 21*** | 213 ± 27*** |
| Metabolic clearance (ml/hr/kg) | 238 ± 36 | 278 ± 42 | 226 ± 12 | 298 ± 42 |
| Plasma [Inulin] (mg/ml) | 0.19 ± 0.02 | 0.23 ± 0.04 | 0.19 ± 0.01 | 0.19 ± 0.01 |
| GFR (ml/hr/kg) | 584 ± 111 | 482 ± 54 | 603 ± 30 | 549 ± 16 |
| % of GHB reabsorbed | 80.3 ± 2.6 | 66.3 ±10.2** | 63.59 ±5.1** | 61.1 ± 4.8** |

Wang et al, Drug Metab Disp 36:2244. 2008

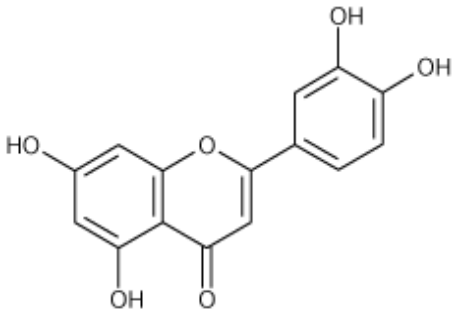
- Luteolin as a MCT inhibitor

# Flavonoids as MCT1 Inhibitors

- Flavonoids phloretin and quercetin are inhibitors of L-lactate transport by MCT1.

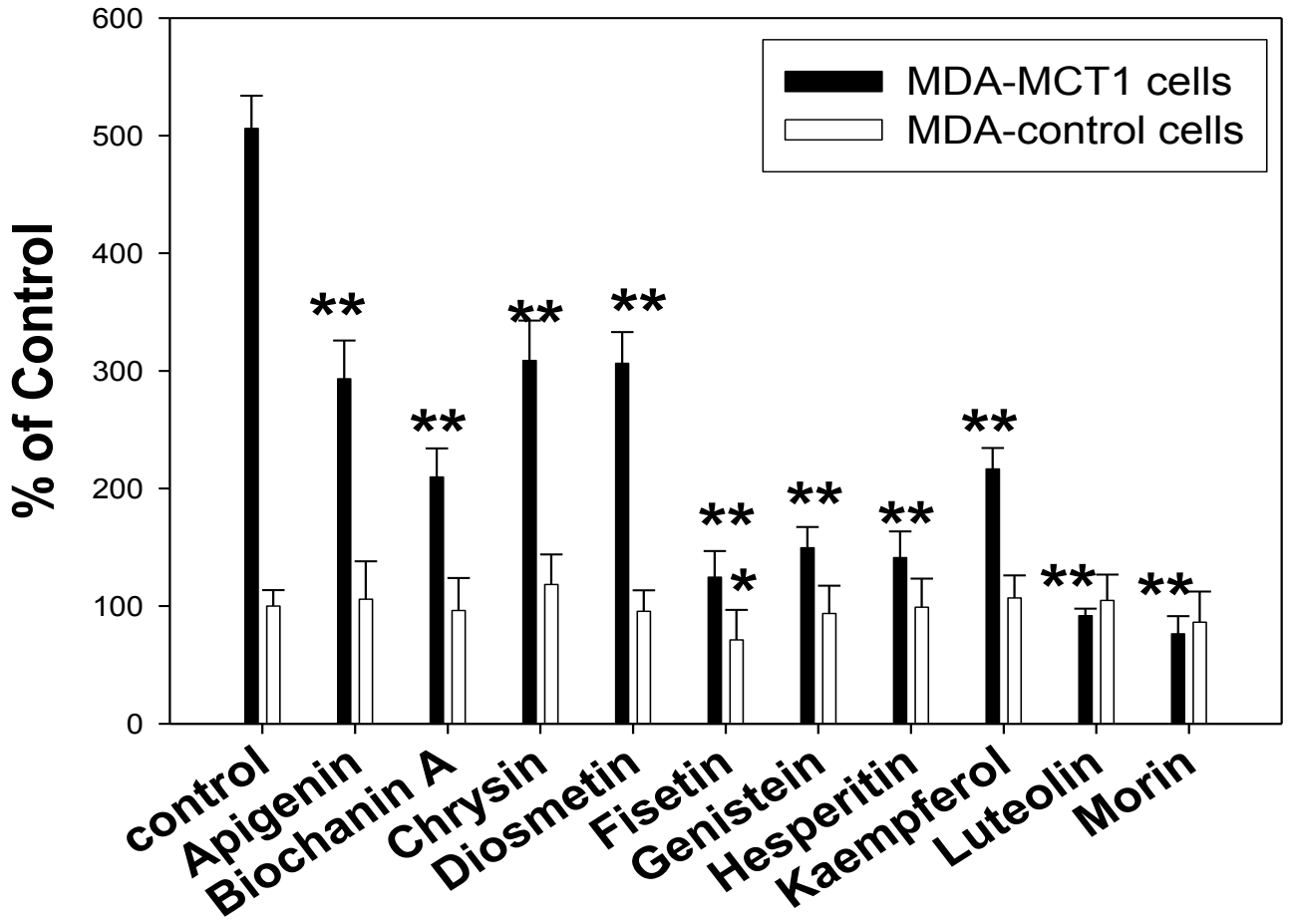



- Flavonoids are inhibitors of various transporters: P-glycoprotein, MRP1, BCRP, OATP




Chemical structure of luteolin

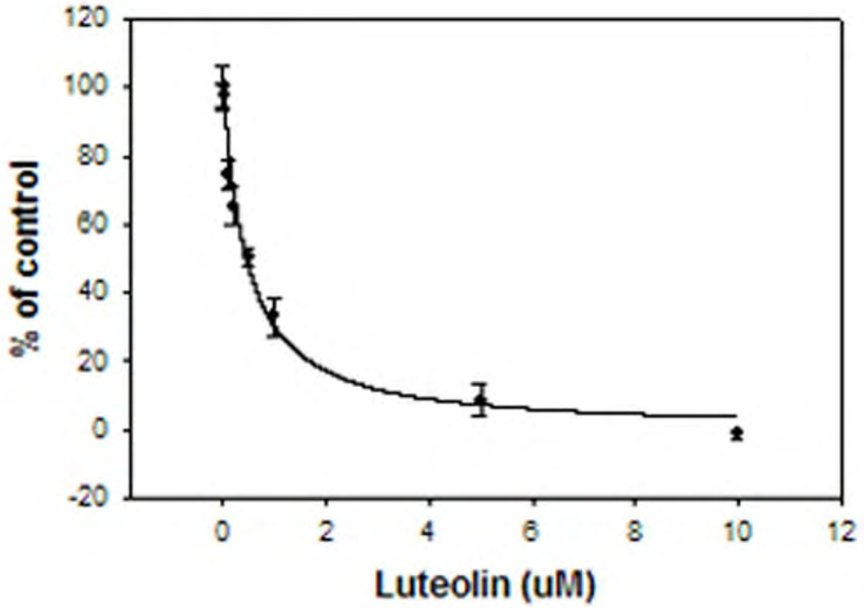
# Flavonoid Inhibition of GHB uptake by MCT1

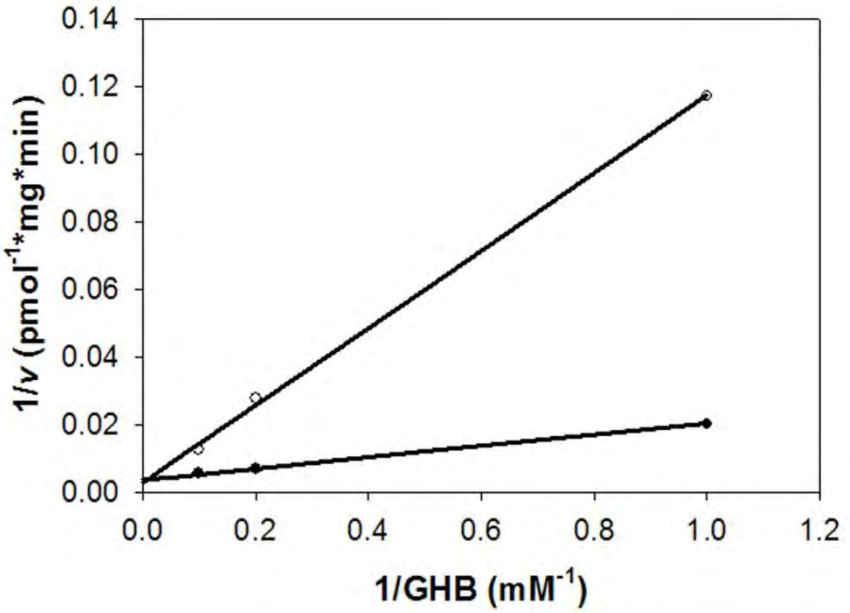



Effects of flavonoids on the uptake of GHB by rMCT1 gene transfected cells and control cells. * $p<0.05$, **$p<0.01$.

Wang and Morris, Drug Metab Dispos. 2007 Feb;35(2):201-8.

# Luteolin is a competitive inhibitor of GHB uptake

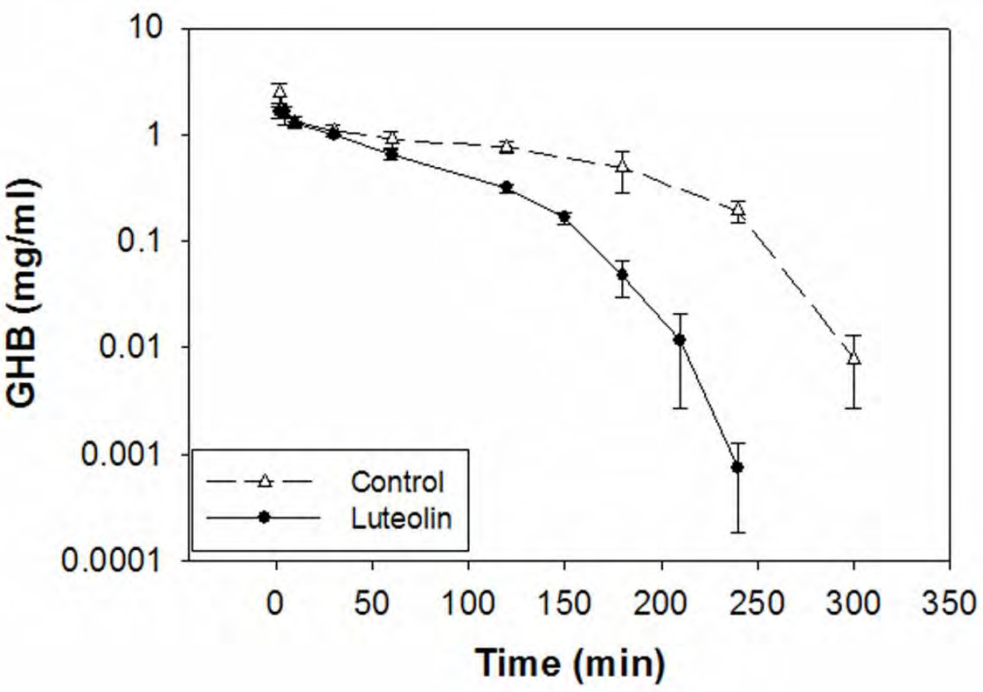



Line-weaver Burk plot. Open circles represent the uptake in the presence of Luteolin (50 μM); closed circles represent the uptake in the absence of Luteolin (50 μM).

| | **Luteolin** | Morin | Phloretin |
|---|---|---|---|
| $IC_{50}$(μM) | **0.41±0.14** | 6.21±2.01 | 2.57±0. 48 |

# Luteolin on GHB TK/TD




| Parameters | Control | Luteolin (10mg/kg) |
|---|---|---|
| AUC (mg/ml*min) | 187 ± 30 | 103 ± 4 ** |
| $\lambda_z$ | 0.056 ± 0.007 | 0.086 ± 0.019 |
| Cl (ml/min/kg) | 5.45 ± 0.91 | 9.76 ± 0.39 ** |
| $V_{ss}$ (ml/kg) | 493 ± 52 | 519 ± 33 |
| Urinary recovery (%) | 25.9 ± 10.4 | 44.1 ± 11.7 |
| $Cl_R$ (ml/min/kg) | 1.36 ± 0.36 | 4.28 ± 1.02** |
| $Cl_m$ (ml/min/kg) | 4.08± 1.15 | 5.48 ± 1.31 |
| Sleep time (min) | 165 ± 10 | 121 ± 5** |

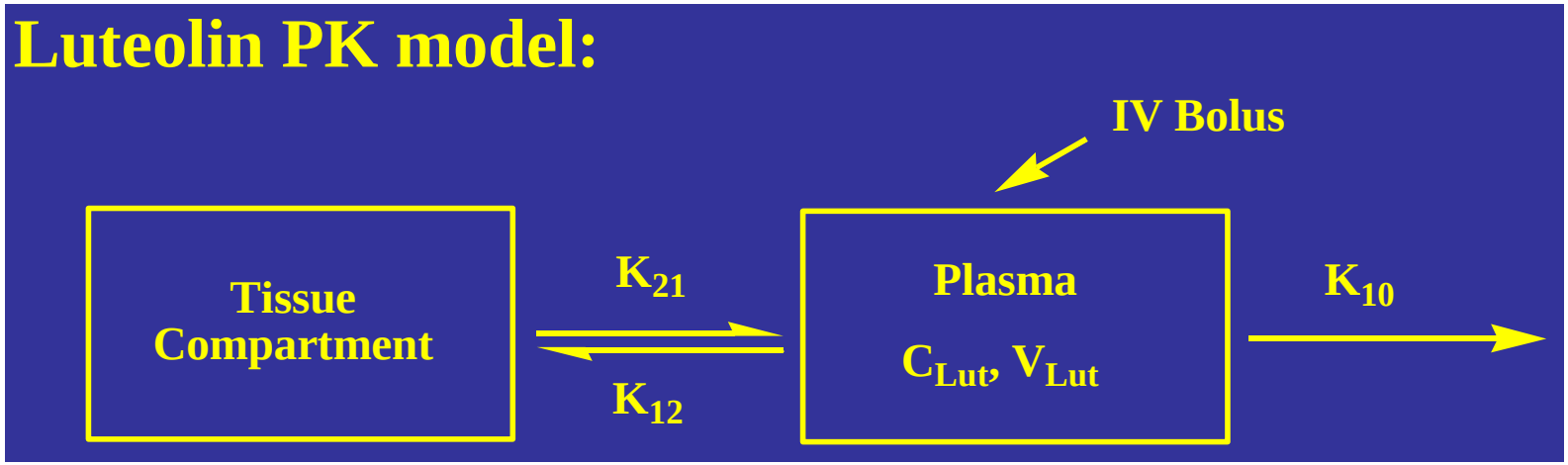
GHB iv bolus 1 g/kg

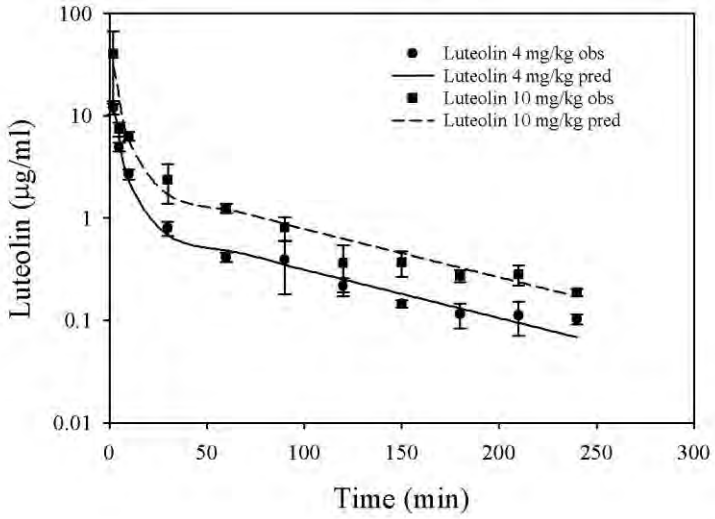
Luteolin iv bolus 10 mg/kg

N = 3 for control and luteolin treatment group

Wang and Morris, Drug Metab Dispos. 2007, 35(2):201-8.

# *Proposed PK Model for Luteolin*

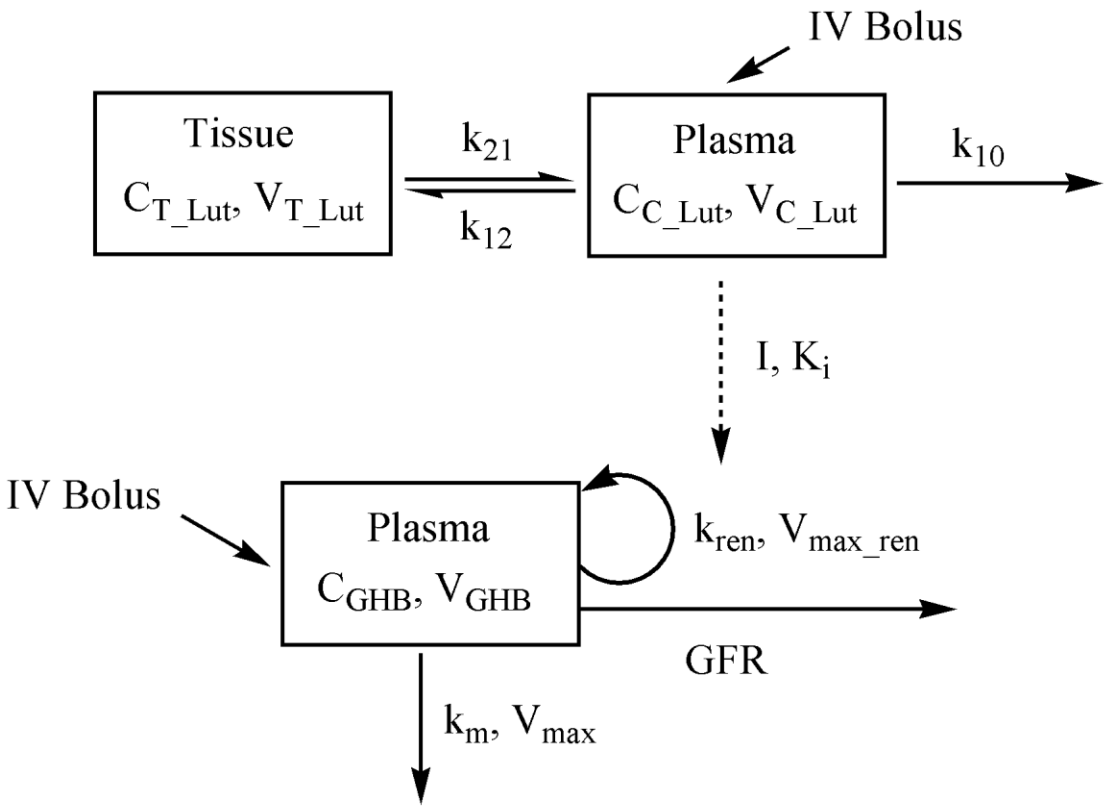



Wang X, Wang X and Morris ME: AAPS J, 2008

# *Proposed PK Model for Luteolin and GHB*

Luteolin PK model:




GHB PK:

- 1 compartment kinetics

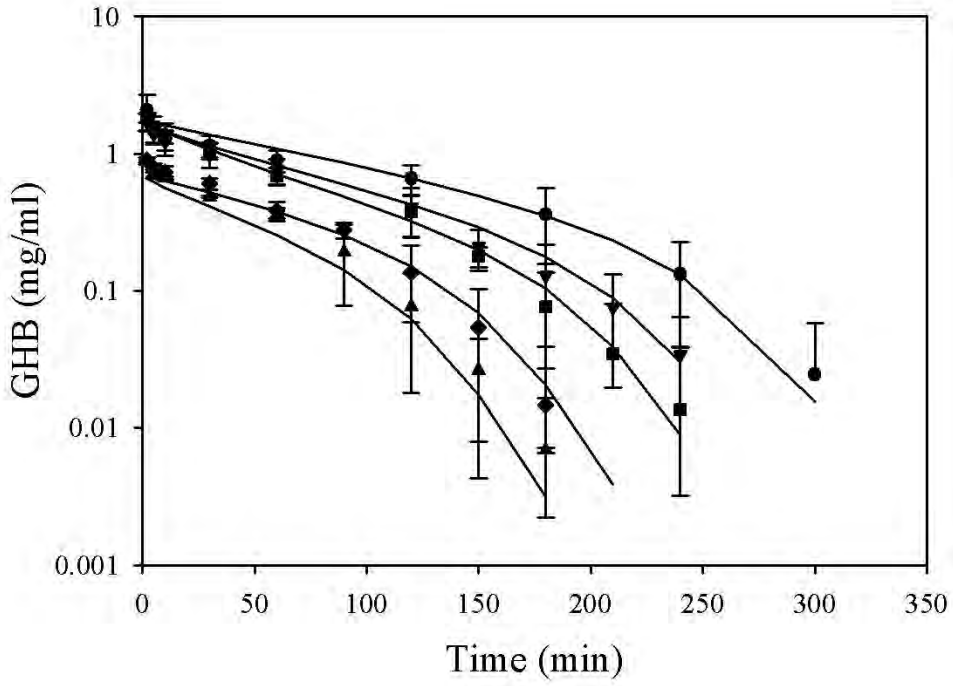
- Nonlinear PK

1) metabolism

2) renal reabsorption.

GHB PK model:

# PK Model Fitting of Luteolin~GHB Interaction




**Luteolin: 0 mg/kg iv**
**2 mg/kg iv**
**4 mg/kg iv**
**10 mg/kg iv**
**20 mg/kg iv**

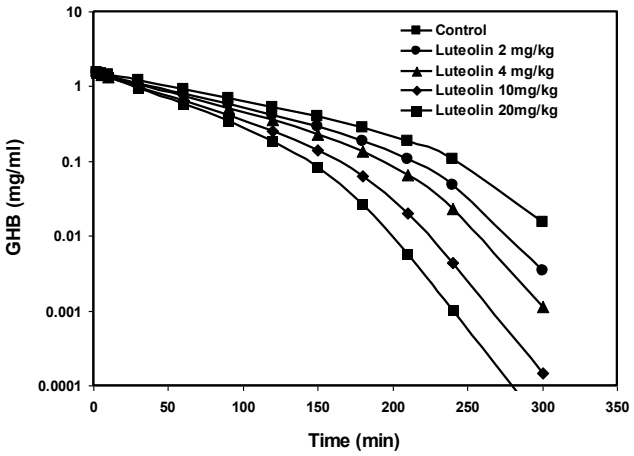
| | Fitted Value | CV % |
|---|---|---|
| $K_m$ (mg/ml) | 0.063 | fixed |
| $V_{max}$ (mg/min/kg) | 2.27 | fixed |
| $V_{C_GHB}$ (ml/kg) | 578 | 3.63 |
| GFR (ml/min/kg) | 10 | fixed |
| $V_{max_ren}$ (mg/min/kg) | 49.2 | 38.5 |
| $K_{ren}$ (mg/ml) | 5.54 | 43.5 |
| Ki' (ug/kg) | 63.3 | 35.2 |

**True Ki = Ki'/$Vc_{_GHB}$ = 1.1 μM**

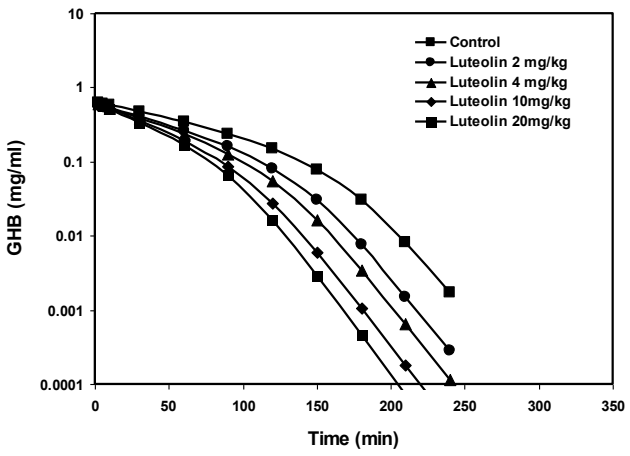
# *Simulation of Luteolin-GHB Interaction*

- To determine the effect of varying doses of luteolin on GHB detoxification at different dose levels of GHB.

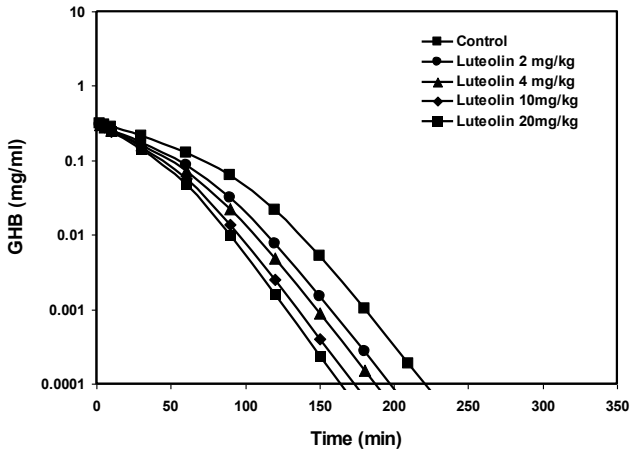
**GHB: 1000 mg/kg iv**




**GHB: 400 mg/kg iv**



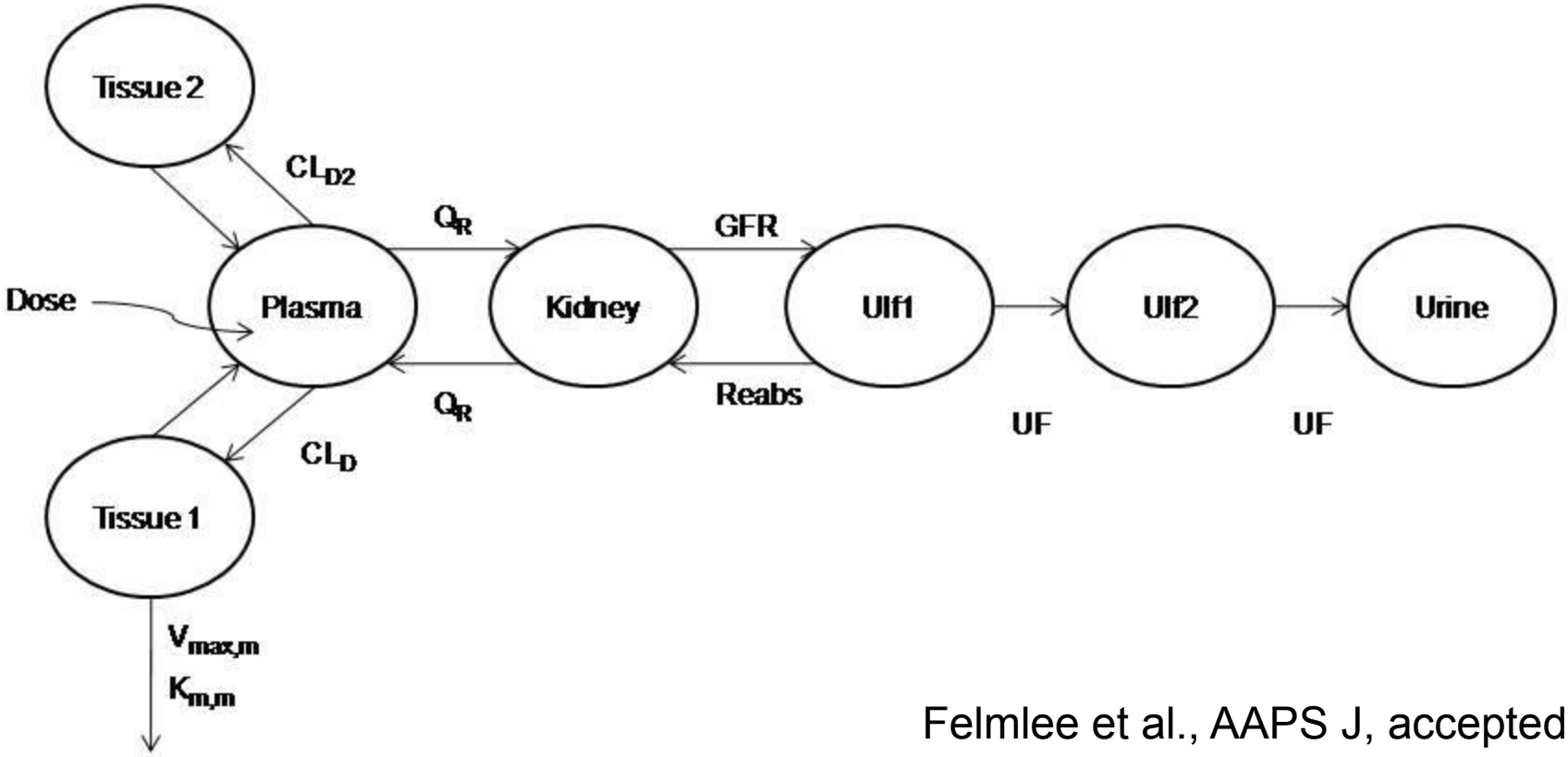

**GHB: 200 mg/kg iv**




**Luteolin: 0 mg/kg iv**
**2 mg/kg iv**
**4 mg/kg iv**
**10 mg/kg iv**
**20 mg/kg iv**

**Simulation by ADAPT II with 10 % Variance**

# Renal Clearance Model

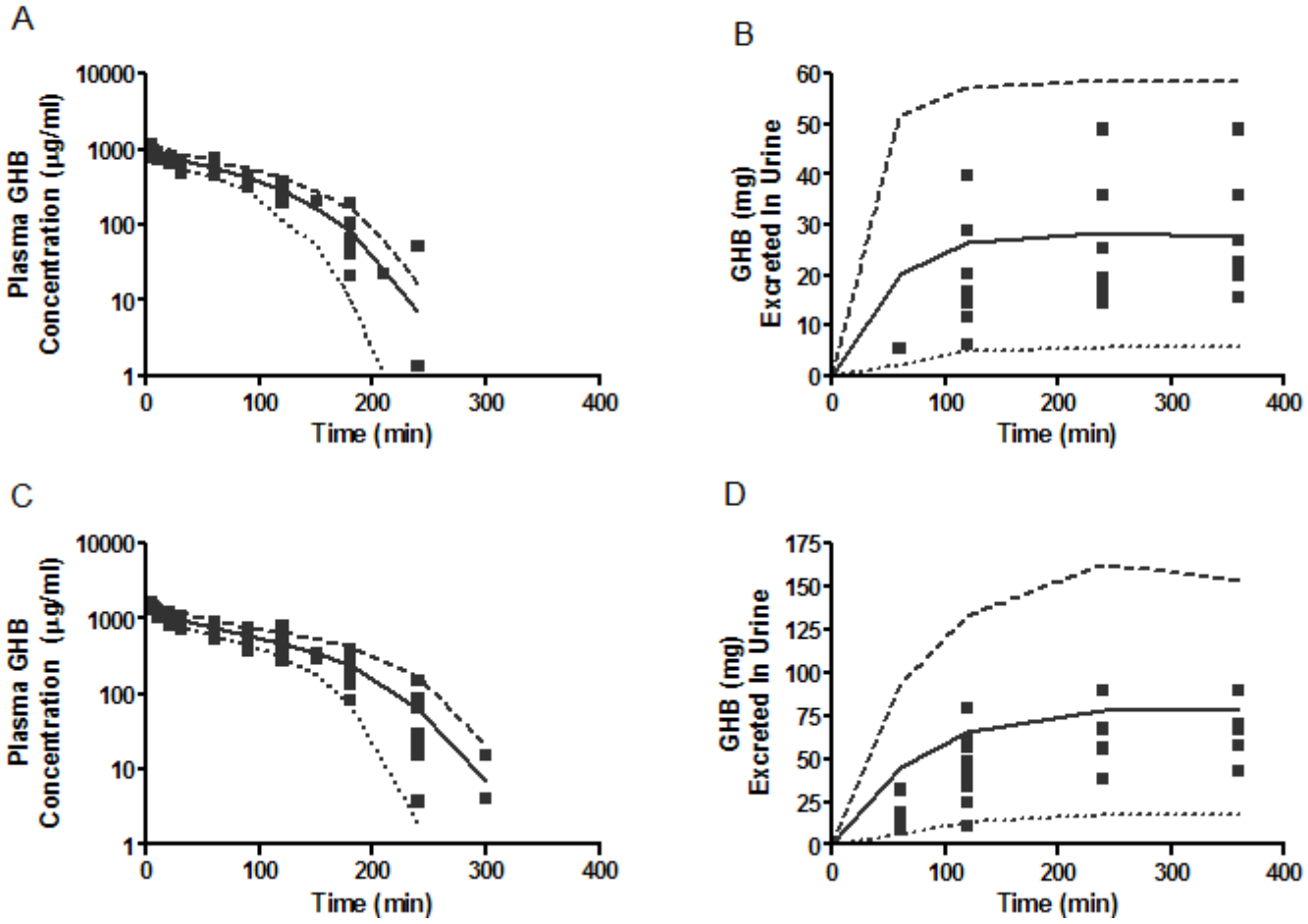



Felmlee et al., AAPS J, accepted

# Mechanistic Model Fitting

400 mg/kg

600 mg/kg




Solid line is population model fitting; dotted are 10$^{th}$ percentile; dashed are 90$^{th}$ percentile.

# MCT1 is a target for immunosuppression

(Murray et al, Nature Chem Biol 2005)

- Novel mechanism of action for immunosuppressant agents through inhibition of MCT1
- Suppression of T-lymphocyte proliferation by inhibiting the MCT-mediated efflux of lactate

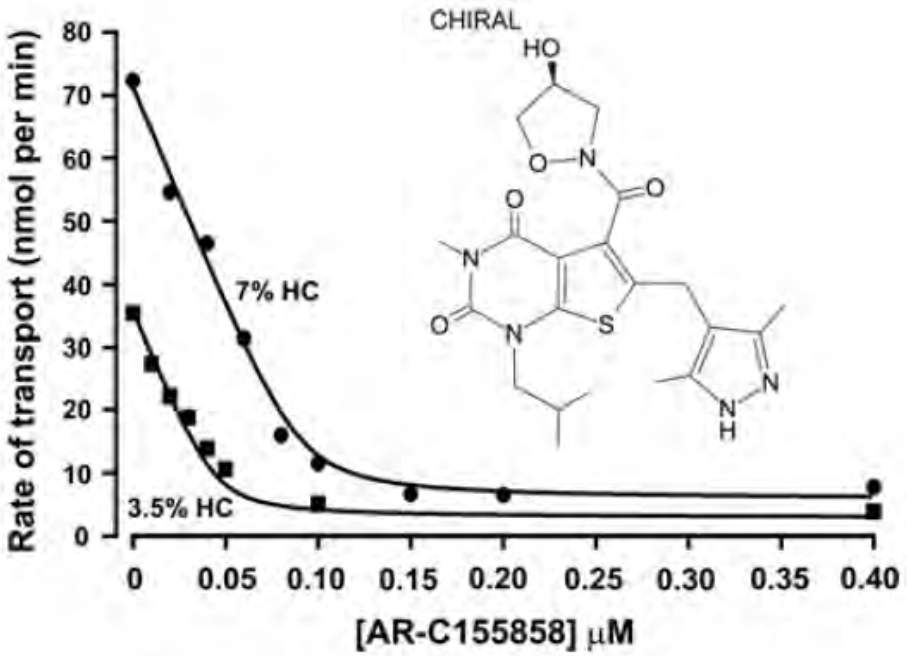


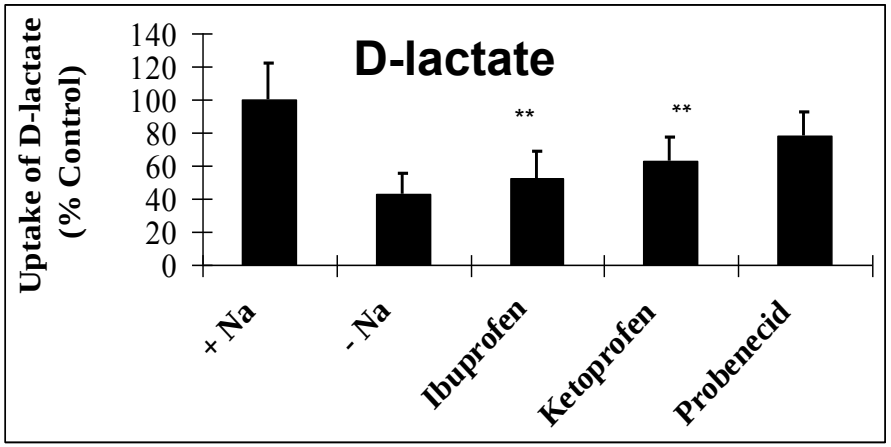

Figure 1 Inhibition of L-lactate uptake into rat erythrocytes by AR-C155858

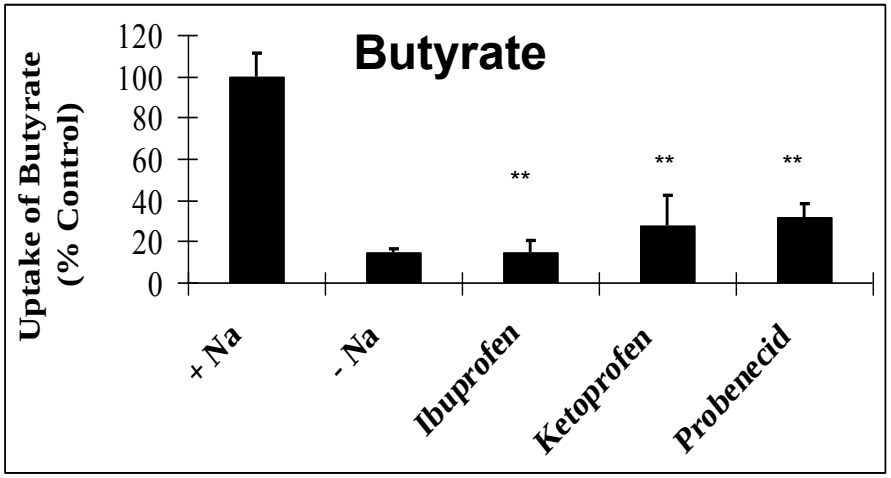
AR-C155858

Ovens et al. Biochem J 425: 523, 2010

# Transport by SMCT1 (SLC5A8)

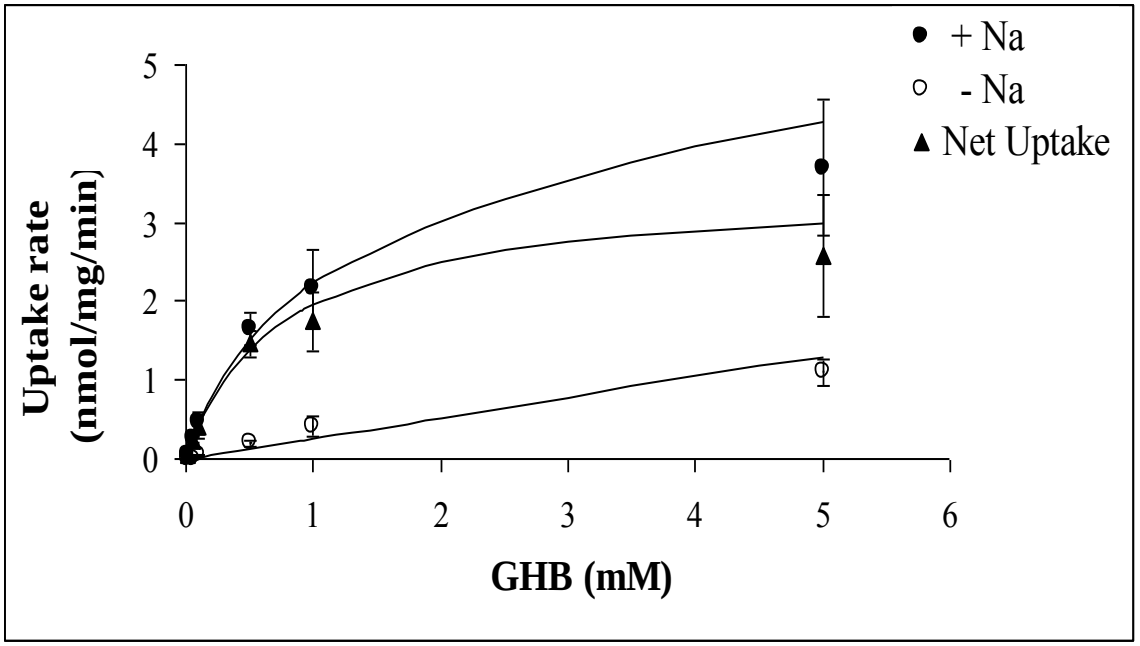
# Effect of inhibitors on the uptake of D-lactate and butyrate in Rat Thyroid FRTL-5 Cells (Cui and Morris, DMD, 2009)




pH7.4, 5min uptake by FRTL-5 at RT, concentration of inhibitors: 1mM
* $p < 0.05$, ** $p < 0.01$

# Concentration dependence of GHB uptake in Rat Thyroid FRTL-5 cells expressing SMCT1




*pH 7.4,*
*5min uptake at RT*

*Eq 1.* $v = \frac{V_{max} \times C}{K_m + C}$ **Net uptake ($Na^+$-$Na^-$)**

*Eq 2.* $v = \frac{V_{max} \times C}{K_m + C} + P \times C$ **+Na**

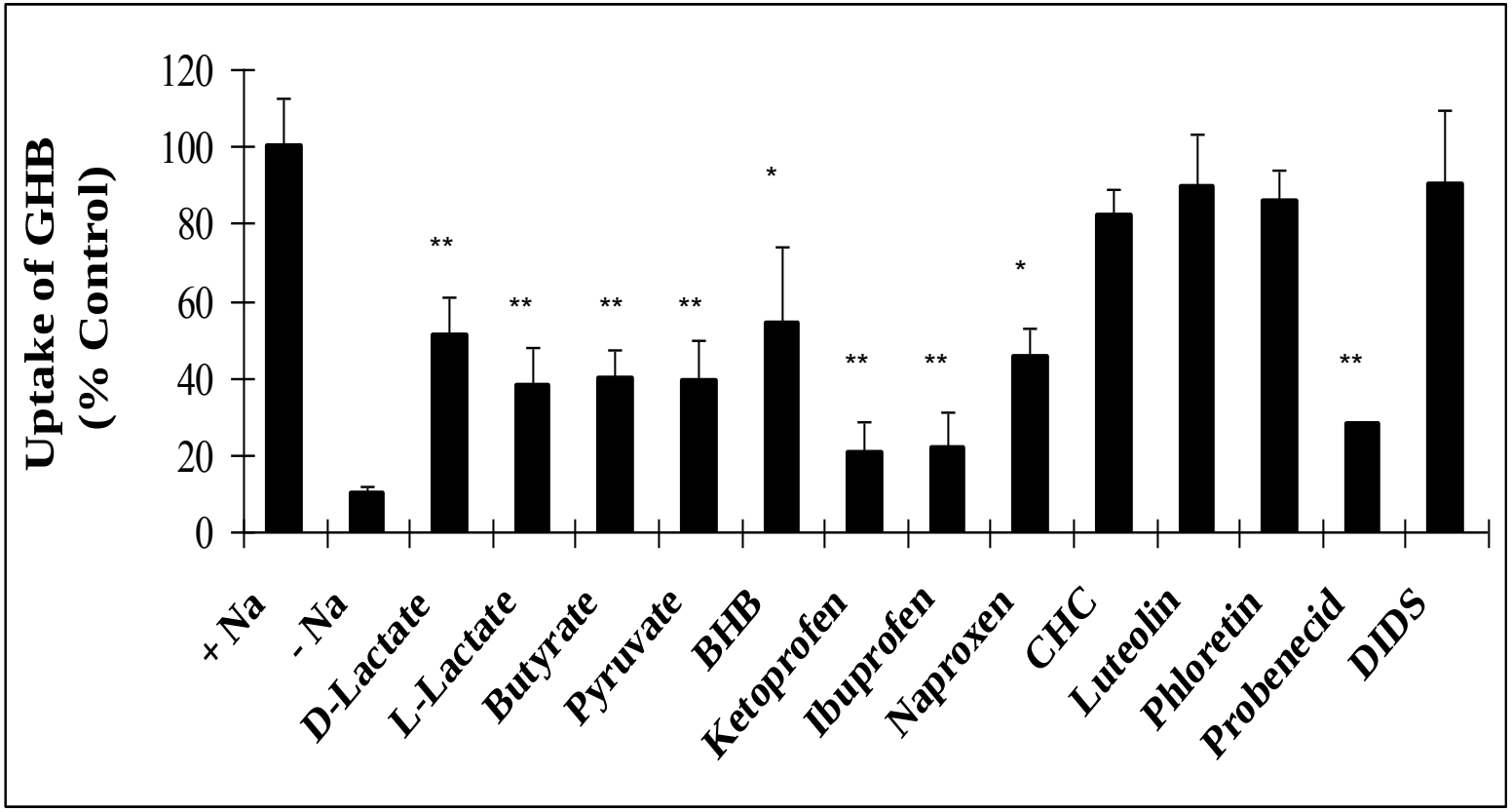
*Eq 3.* $v = P \times C$ **- Na**

*Eq 4.* $v = \frac{V_{max\,1} \times C}{K_{m1} + C} + \frac{V_{max\,2} \times C}{K_{m2} + C}$

Kinetic parameters for GHB uptake in FRTL-5 cells

| Parameters | Values |
|---|---|
| $K_m$ (mM) | 0.74 ± 0.30 |
| $V_{max}$ (nmol·mg$^{-1}$·min$^{-1}$) | 3.47 ± 1.43 |
| P (µl mg$^{-1}$·min$^{-1}$) | 0.24 ± 0.11 |

# Effect of potential inhibitors on the sodium-dependent uptake of GHB



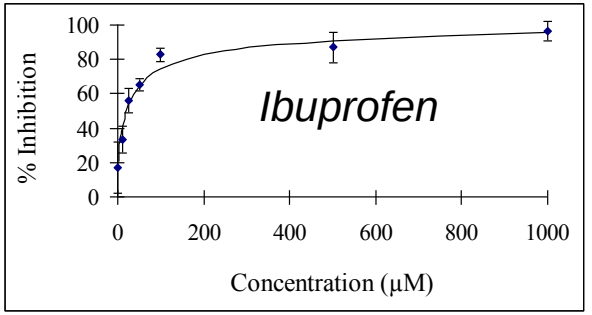

pH 7.4, GHB 10μM, 5min uptake by FRTL-5 at RT
Concentration of inhibitors: phloretin 0.25mM, luteolin 0.05mM, all others 1mM
* $p < 0.05$, ** $p < 0.01$

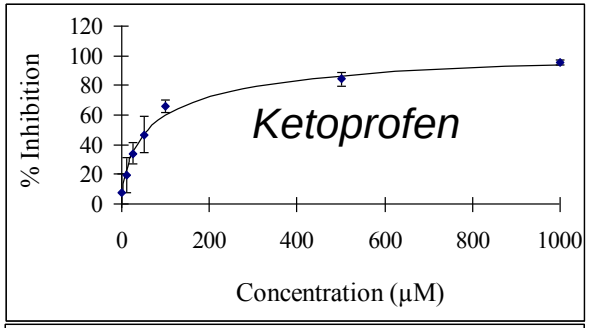
# $IC_{50}$ values for the inhibition of GHB

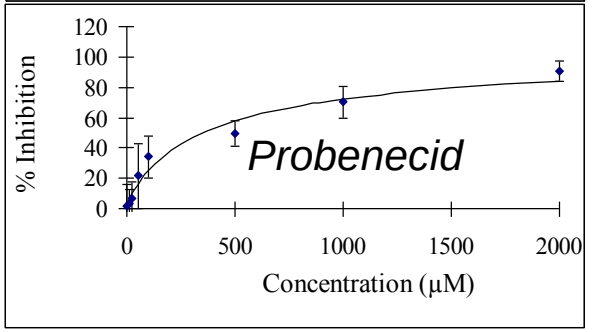
| Inhibitors | $IC_{50}$ (μM) |
|---|---|
| Ibuprofen | 31.6 ± 19.7 |
| Ketoprofen | 64.4 ± 23.4 |
| Probenecid | 379.7 ± 43.4 |
| L-lactate | 100.5 ± 37.3 |

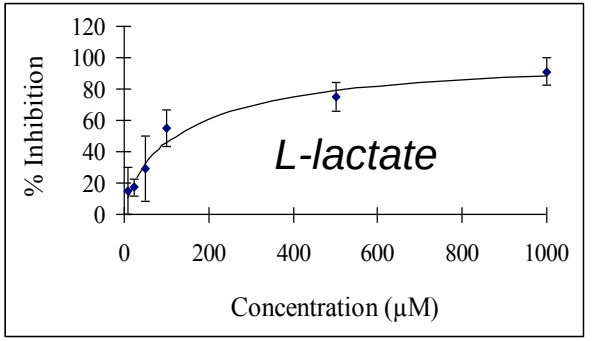
$$\%\ Inhibition\ (F) = \left( \frac{v_{Na^+} - v_{inh}}{v_{Na^+} - v_{Na^-}} \right) \times 100$$

$$F = \frac{I_{\max 1} \times C^{\mathrm{r}}}{IC_{50}{}^{r} + C^{r}} \times 100$$




pH7.4, GHB 10 μM,
5min uptake by FRTL-5 at RT

# Summary

- GHB is a substrate for MCT1, 2 and 4, and for SMCT1.
- Reabsorption of GHB in the renal proximal tubule can be inhibited by MCT inhibitors, resulting in increased renal and total clearances.
- Currently, we are assessing the importance of MCTs in tissue distribution, including distribution into the brain
- Inhibition of MCT-mediated transport (renal/brain)may represent a potential strategy for treating overdoses of this drug of abuse.

# Acknowledgements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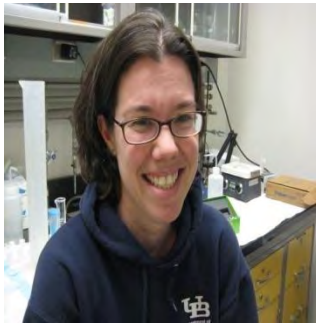

Melanie Felmlee

Samuel Roiko

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