The Challenges of Designing Cyclic Prodrugs of Opioid Peptides that Permeate the Intestinal Mucosa and the Blood-Brain Barrier

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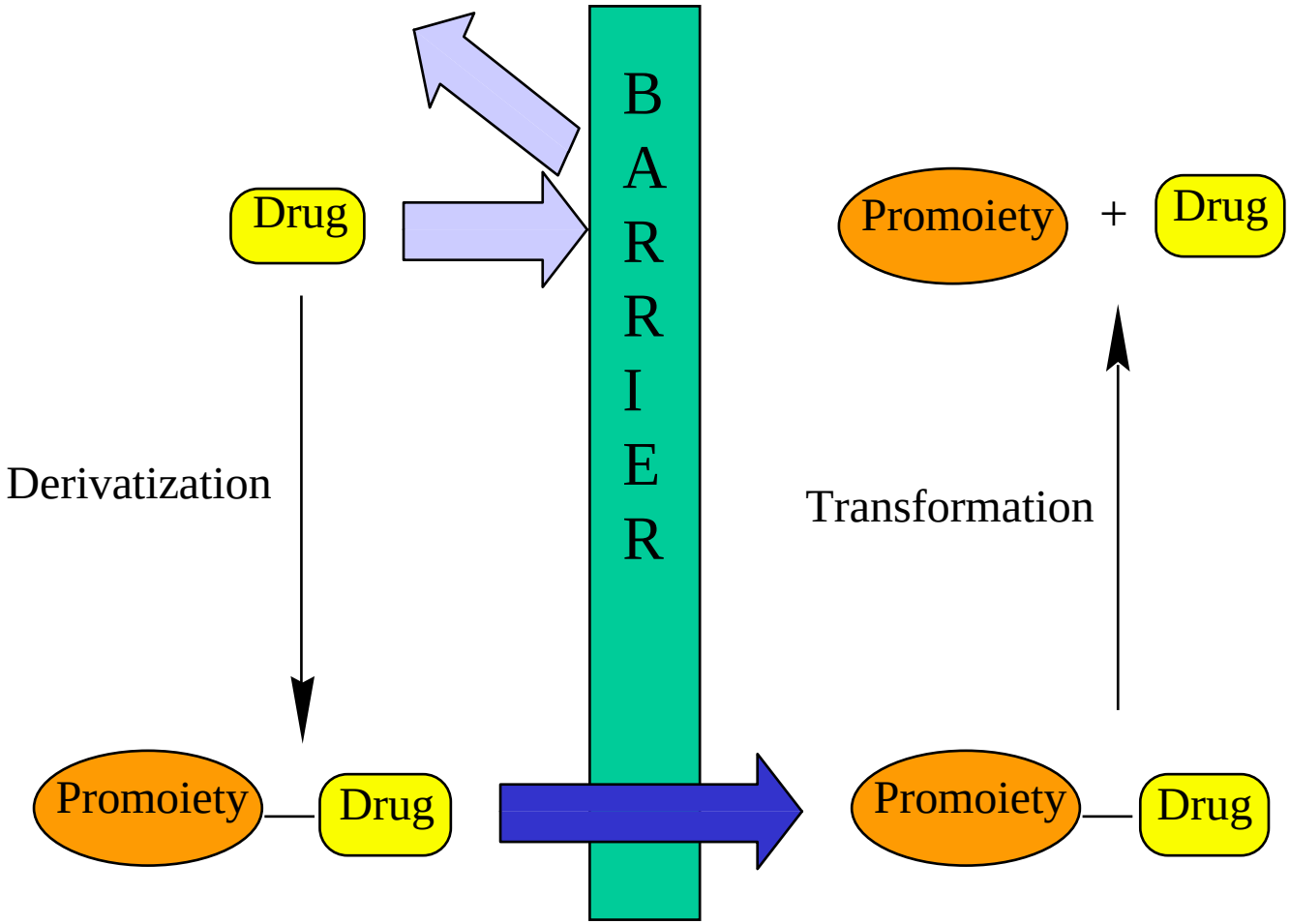
Prodrug Strategy



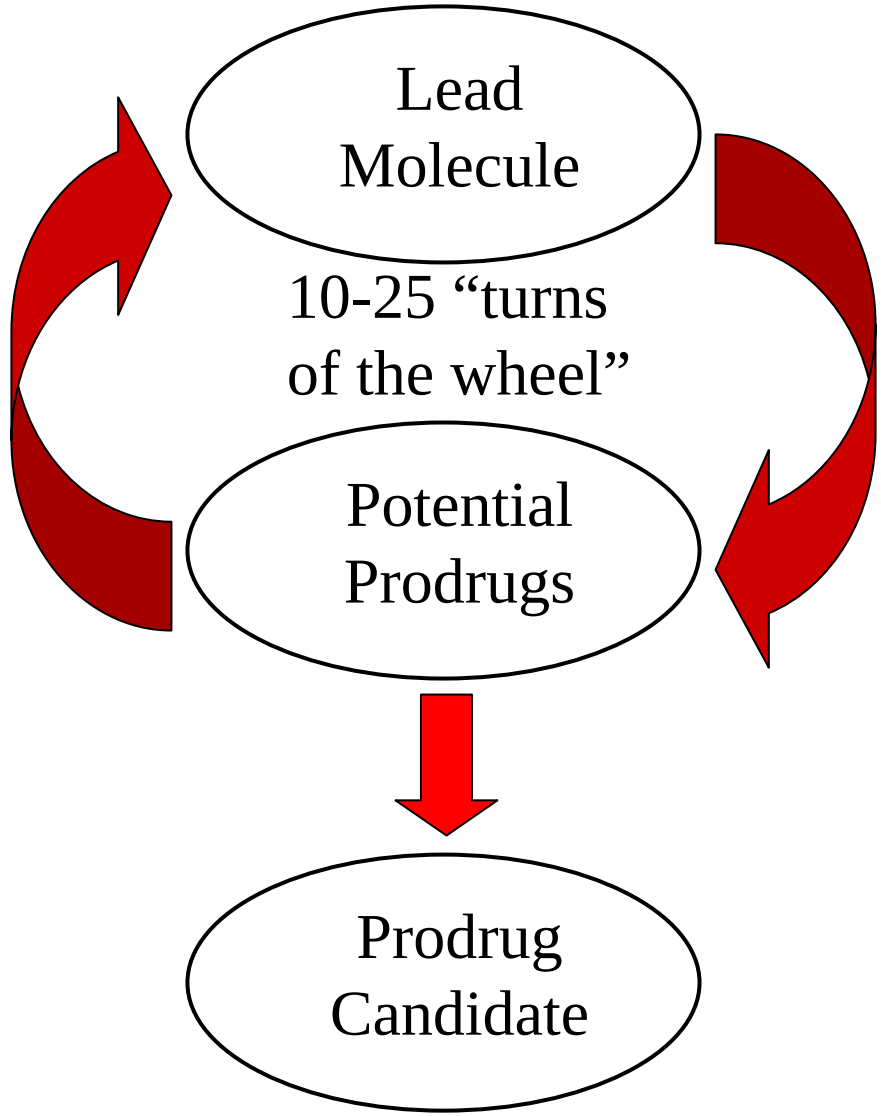
Reference: Prodrugs: Challenges and Rewards (V. Stella, R. T. Borchardt, M. Hageman, R. Oliyai, J. Tilley and H. Maag, Eds Springer, New York, NY, 2007).

Barriers

- **Solubility**
- **Stability**
- **Permeability**
- **Presystemic Metabolism**

Reference: Prodrugs: Challenges and Rewards (V. Stella, R. T. Borchardt, M. Hageman, R. Oliyai, J. Tilley and H. Maag, Eds Springer, New York, NY, 2007).

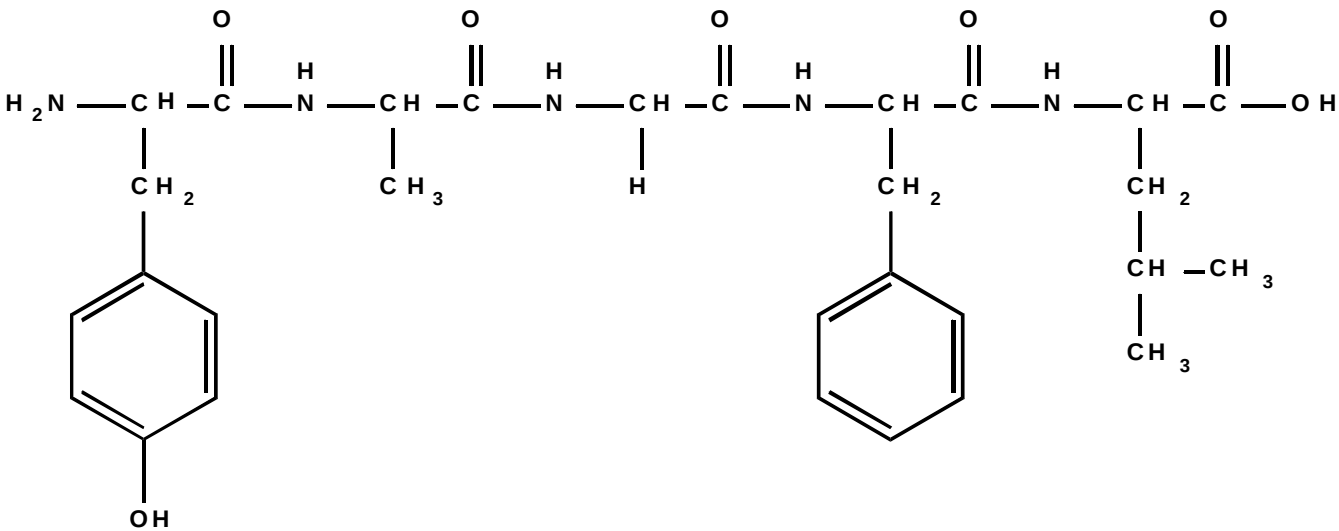
The Iterative Process is Crucial to the Discovery of Prodrugs!!!!!!



Design of new potential prodrugs .

in vitro and **<u>extensive</u>** *in vivo* testing of ADME properties

Case History

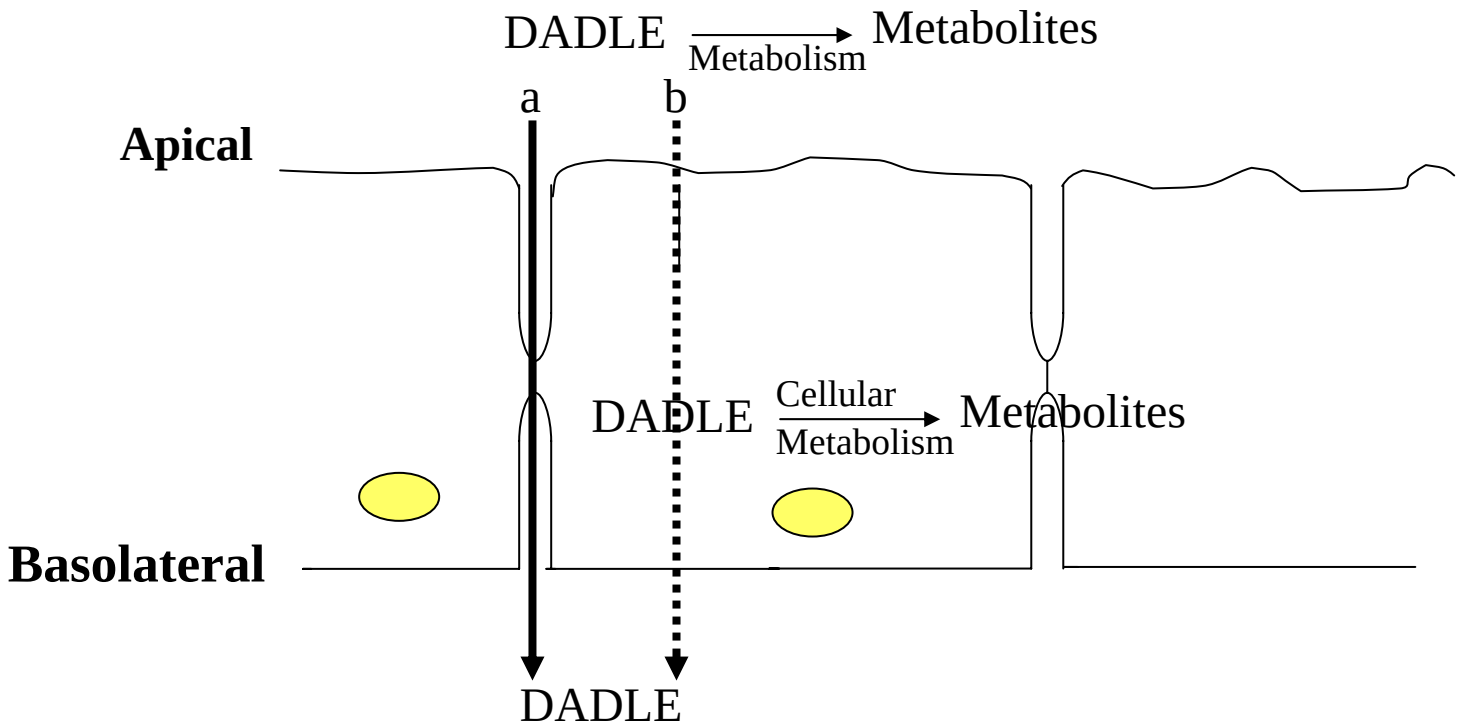


H-Tyr-D-Ala-Gly-Phe-D-Leu-OH

DADLE

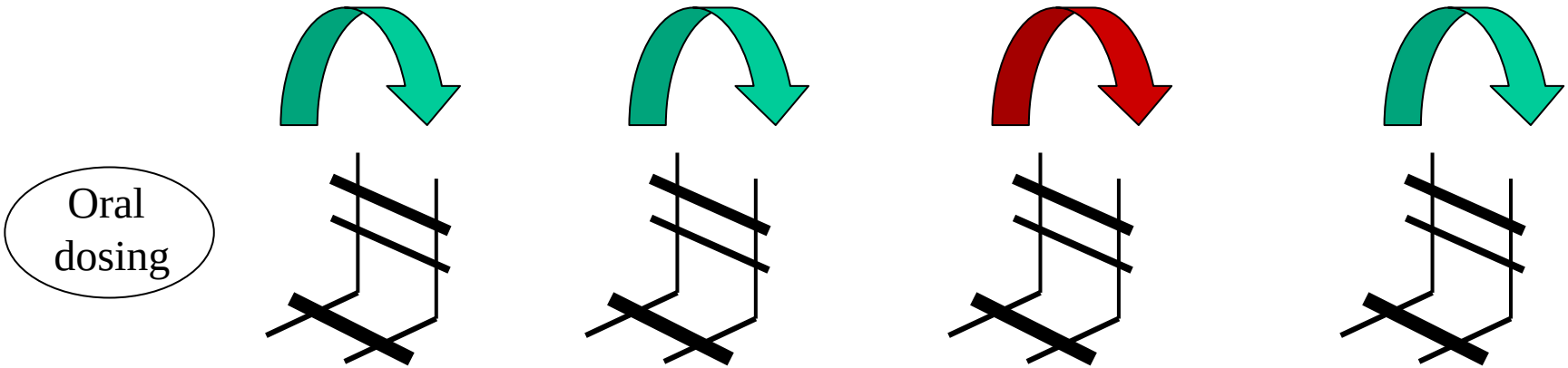
Project Goals: To enhance the oral bioavailability and BBB permeation of this opioid peptide.

DADLE exhibits low permeation across the intestinal mucosa and the BBB because it is a paracellular permeant and it is metabolically labile.



a, paracellular diffusion
b, transcellualr diffusion

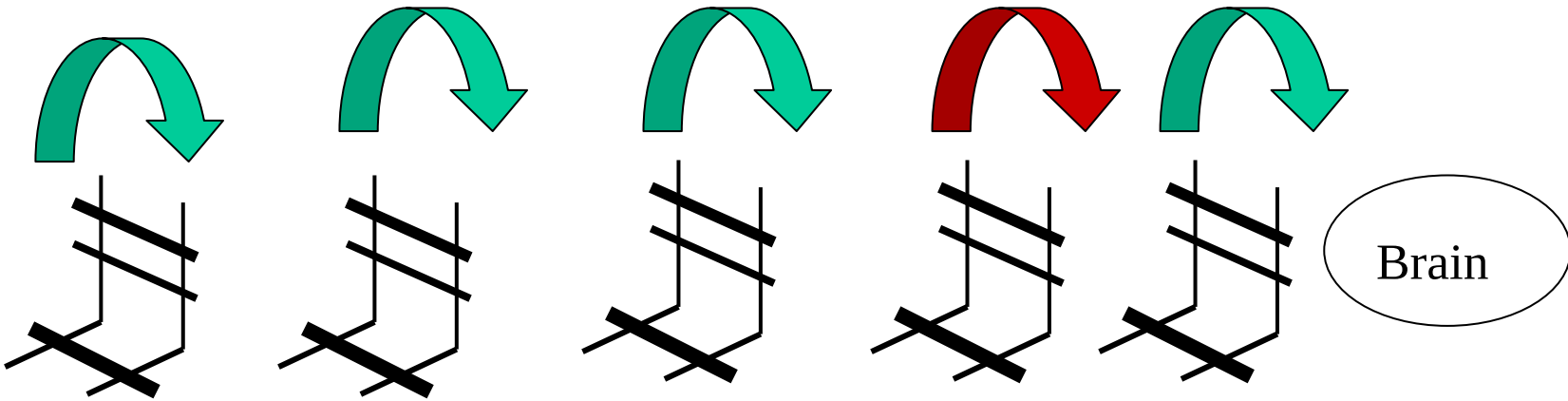
Key Biopharmaceutical Properties of an Orally Active Prodrug Targeted to the Brain



	Solubility	Bioconversion in Intestinal Lumen	Intestinal Mucosal Permeation	Bioconversion in Intestinal Mucosa
Desirable Characteristics	MED/HIGH	LOW	**HIGH**	LOW

Reference: Prodrugs: Challenges and Rewards (V. Stella, R. T. Borchardt, M. Hageman, R. Oliyai, J. Tilley and H. Maag, Eds, Springer, New York, NY, 2007).

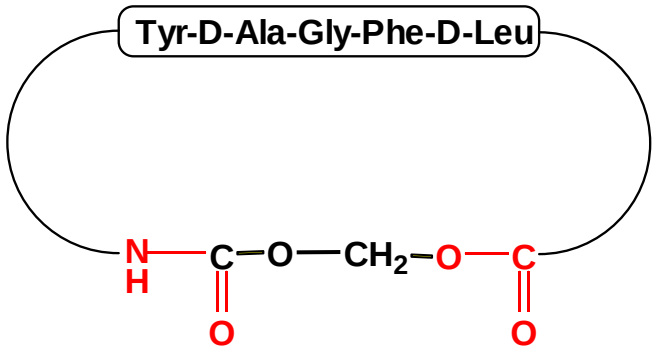
Key Biopharmaceutical Properties of an Orally Active Prodrug Targeted to the Brain, Cont'd



	Bioconversion in Blood	Liver, Kidney Clearance	Protein Binding	BBB Permeation	Bioconversion in Brain
Desirable Characteristics	LOW	LOW	LOW/MED	**HIGH**	HIGH

Reference: Prodrugs: Challenges and Rewards (V. Stella, R. T. Borchardt, M. Hageman, R. Oliyai, J. Tilley and H. Maag, Eds, Springer, New York, NY, 2007).

Cyclic Prodrugs of DADLE

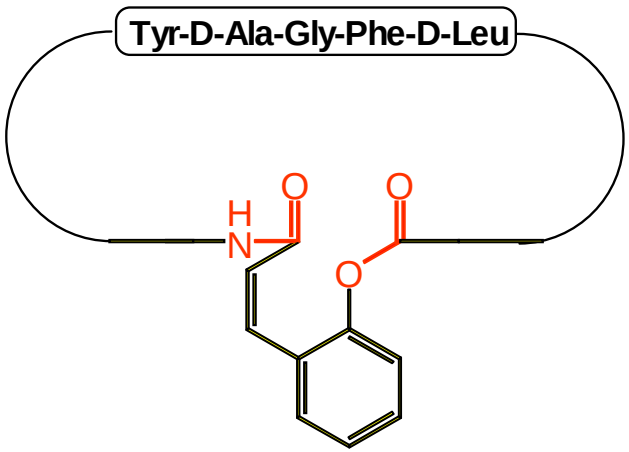


Acyloxyalkoxy-based cyclic prodrug

(AOA-DADLE)

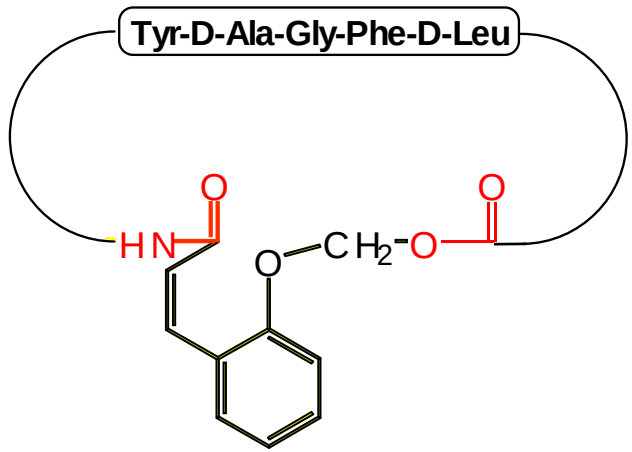
References:

- **Wang *et al.*, *J. Peptide Res.*, 53, 370-392, 1999**
- **Bak *et al.*, *J. Peptide Res.*, 53, 393-406, 1999**
- **Ouyang *et al.*, *J. Peptide Res.*, 59,183-195, 2002**



Coumarinic acid-based cyclic prodrug

(CA-DADLE)

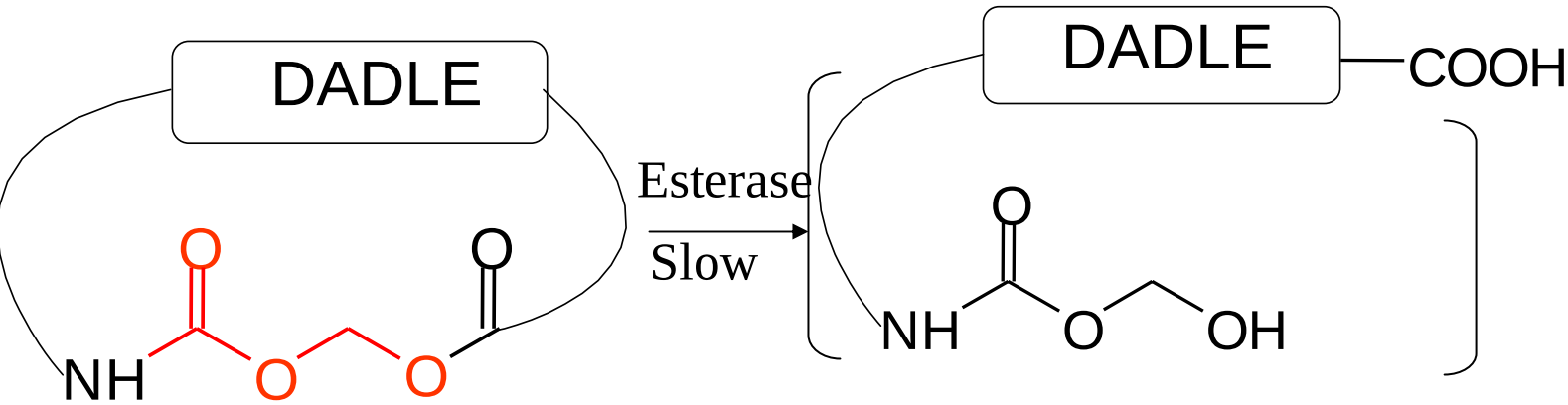


Oxymethyl-modified
Coumarinic acid-based cyclic prodrug

(OMCA-DADLE)

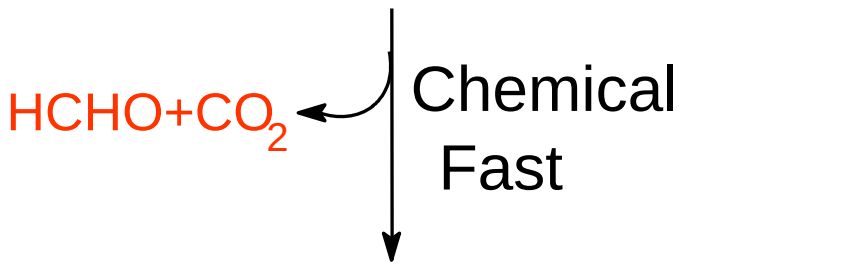
Acyloxyalkoxy (AOA)-Based Cyclic Prodrug Strategy for Improving the Cell Permeation of DADLE

Reference: A. Bak *et al.*, *Pharm. Res.*, 16, 24-29, 1999



AOA-DADLE

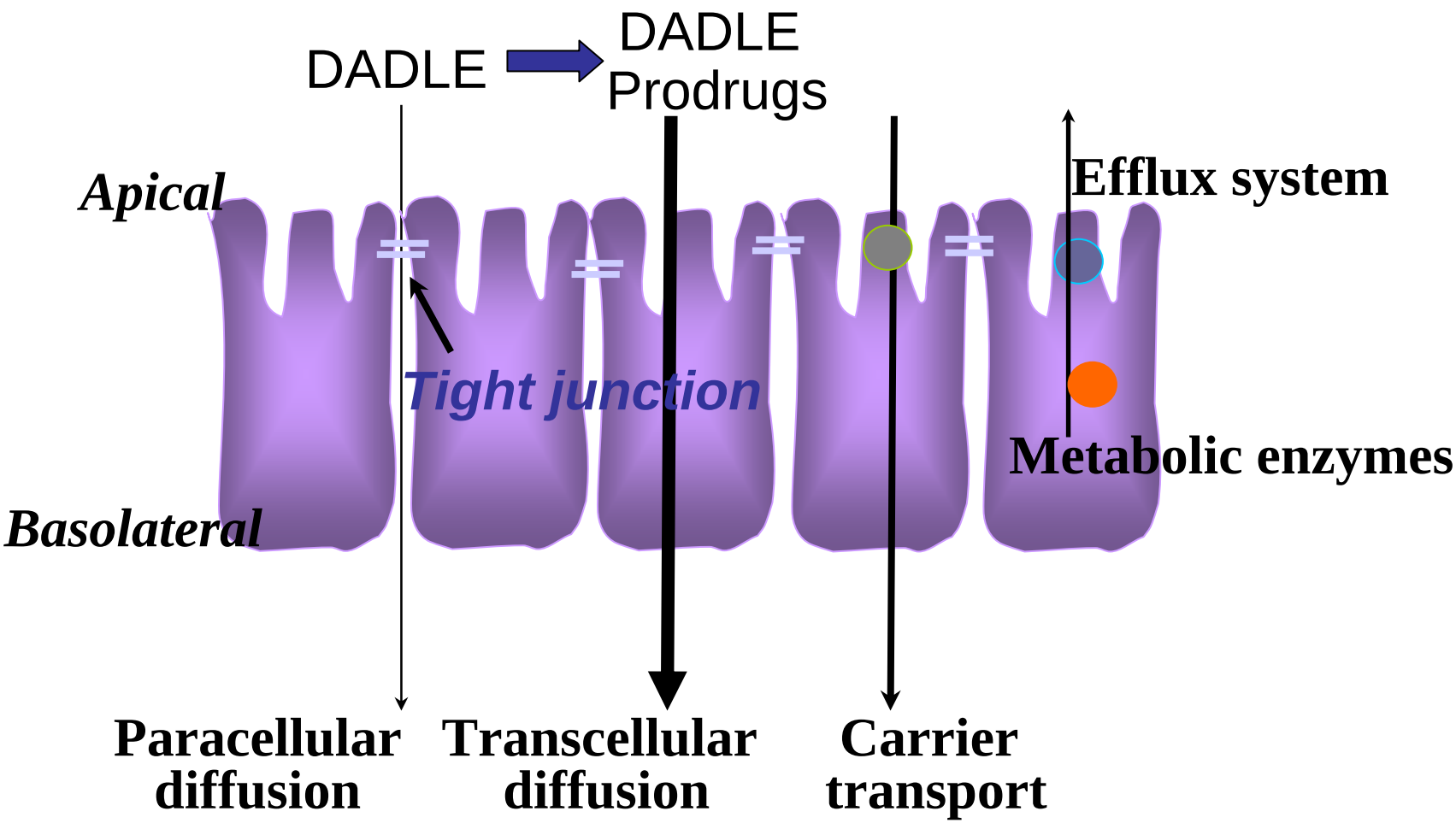
Hydrophobic
(Transcellular Permeant)



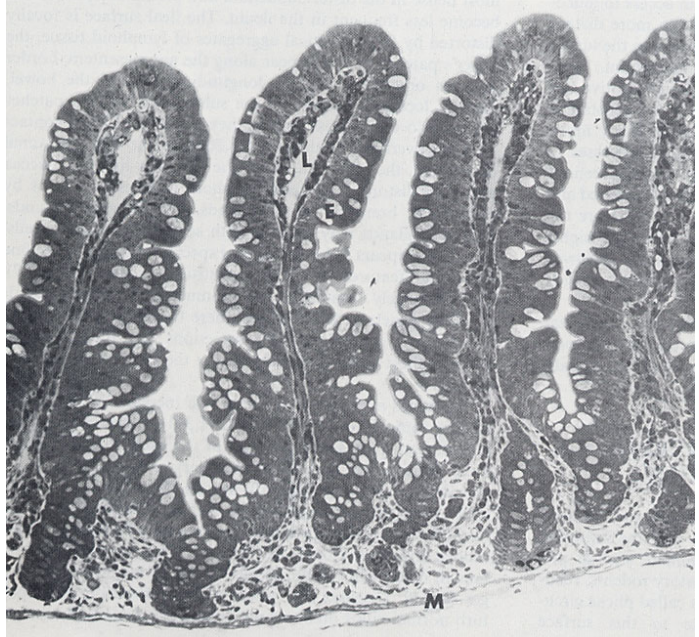
Hydrophilic
(Paracellular Permeant)

Predicted Permeabilities:
AOA-DADLE>>>>DADLE

Prodrugs were designed to be transcellular permeants of the intestinal mucosa.

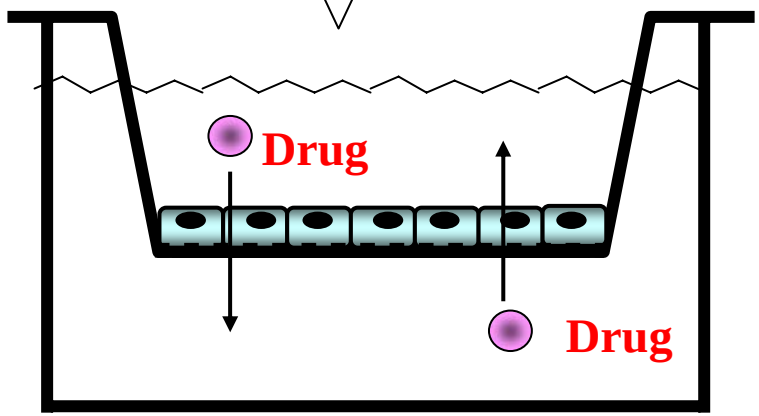


The concept of using cell culture techniques to determine intestinal mucosal permeation was introduced into the pharmaceutical sciences in the late 1980s (Hidalgo, Raub and Borchardt, Journal of Gastroenterology, *96*, 609-616, 1989.



Intestinal Epithelium

Cell/Tissue Culture Techniques



Caco-2 cell Monolayers

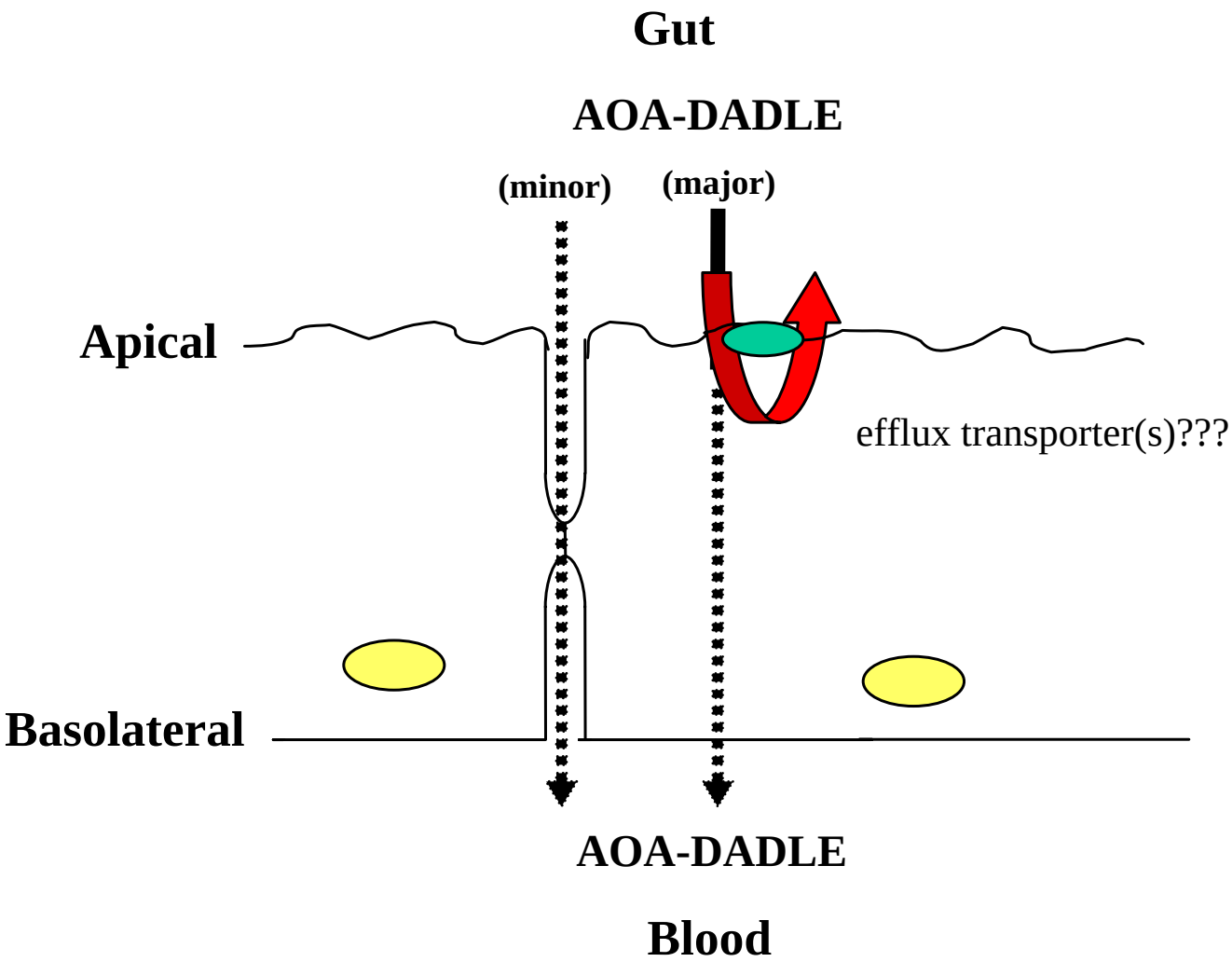
Comparison of the Permeability Coefficients of DADLE and AOA-DADLE in Caco-2 Cells

Reference: A. Bak *et al*., *Pharm. Res*., 16, 24-29, 1999

	Papp*10^{-6} cm/s	**Relative Difference in Permeability**
DADLE	0.078±0.007	**1**
AOA-DADLE	0.0186±0.009	**0.23**

Conclusion: DADLE is more permeable than AOA-DADLE???

Are Efflux Transporters (MDR1, MRP2, BCRP) Restricting the Cell Permeation of AOA-DADLE?



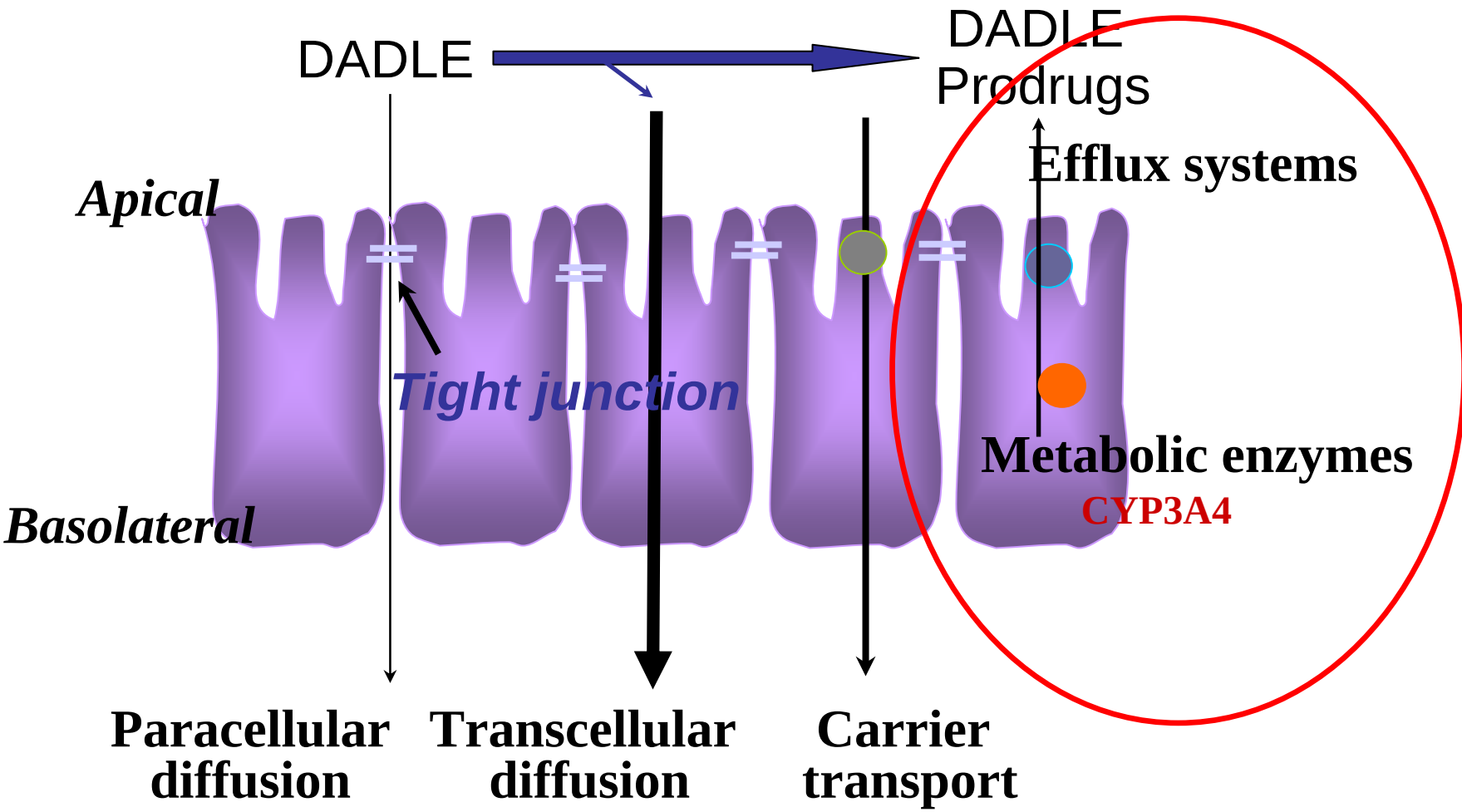
Does AOA-DADLE Exhibit Polarized Efflux in Caco-2 Cells?

Reference: A. Bak et al., *Pharm. Res.*, 16, 24-29, 1999

Permeability	Papp*10^{-6} cm/s	**Ratio of $P_{B\ to\ A}/P_{A\ to\ B}$**
AP to BL	0.0186±0.009	**52**
BL to AP	0.969±0.05	

Conclusion: The low AP to BL permeability of AOA-DADLE results from its substrate activity for an efflux transporter(s) in Caco-2 cells

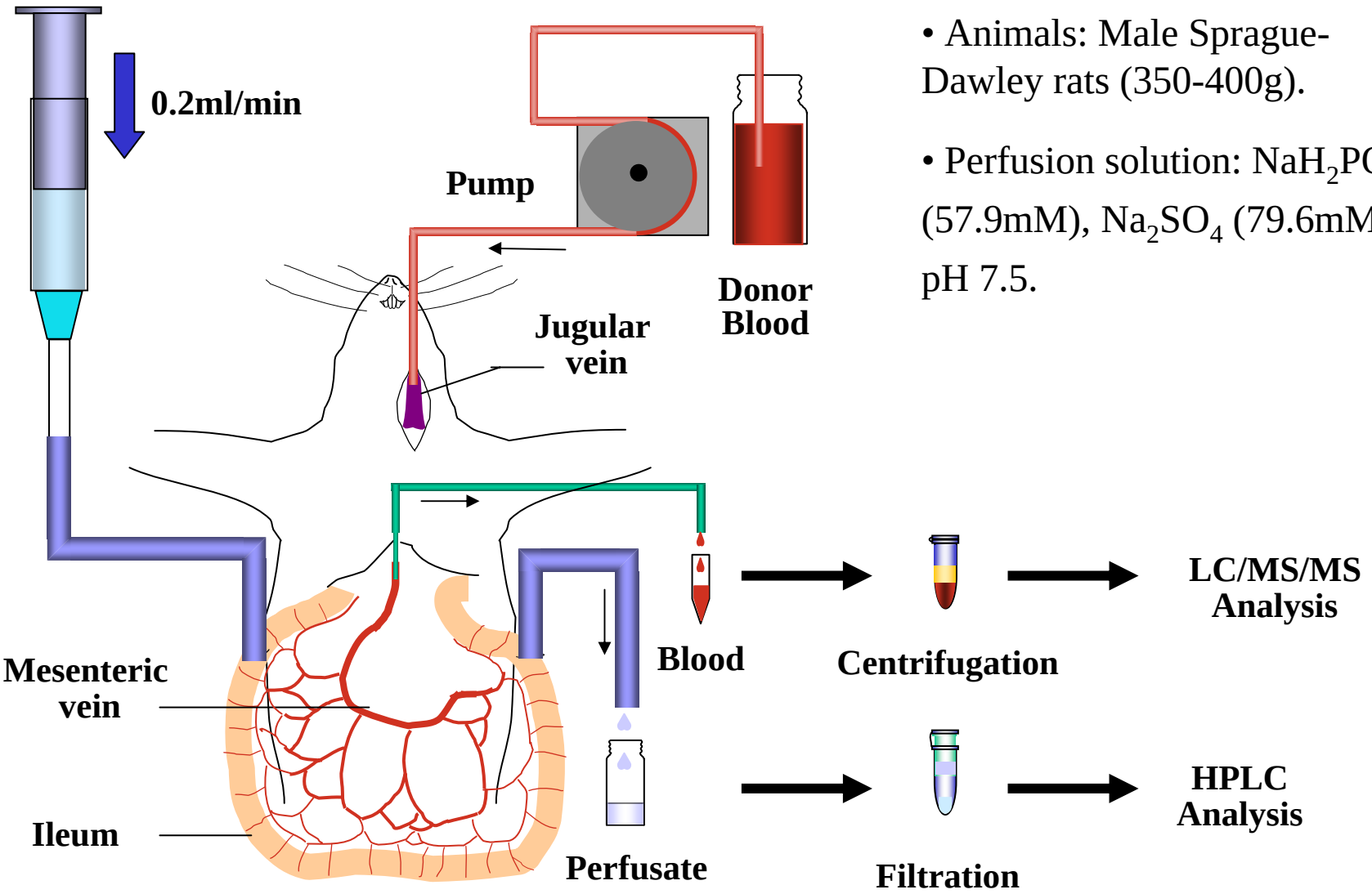
Prodrugs were transcellular permeants but they are also substrates for efflux transporters.



Question: Based on knowledge about the role of efflux transporters in the intestinal mucosa, have we learned anything from these cell permeation experiments that would help predict the oral absorption of these DADLE prodrugs in animals or man?

Answer: Perhaps, depends on the intrinsic permeability of the prodrugs, their solubility, and substrate kinetic parameters for the efflux transporters.

In Situ Rat Ileum Perfusion



- Animals: Male Sprague-Dawley rats (350-400g).
- Perfusion solution: NaH_2PO_4 (57.9mM), Na_2SO_4 (79.6mM), pH 7.5.

Intestinal Mucosal P_B Values of DADLE and Its Prodrugs in the Absence and Presence of a Pgp Inhibitor (PSC833).

Reference: Ouyang *et al.*, *J. Pharm. Sci.*, 98, 337-348, 2009.

Compound	$P_B \times 10^8$ (cm/sec)		
	-inhibitors	+PSC	Relative Increase in P_B
DADLE	24.6±6.4	—	—
AOA-DADLE	4.0 ± 1.7	162 ± 36	**40.5**
CA-DADLE	5.6± 2.5	177 ± 56	**31.6**
OMCA-DADLE	4.2± 1.0	189 ± 27	**45**

Results are mean ± SEM, n>3.

P_B: Apparent permeability coefficient based on appearance of drug prodrugs in the blood.

PSC: 10 μM PSC 833, a cyclosporin analog.

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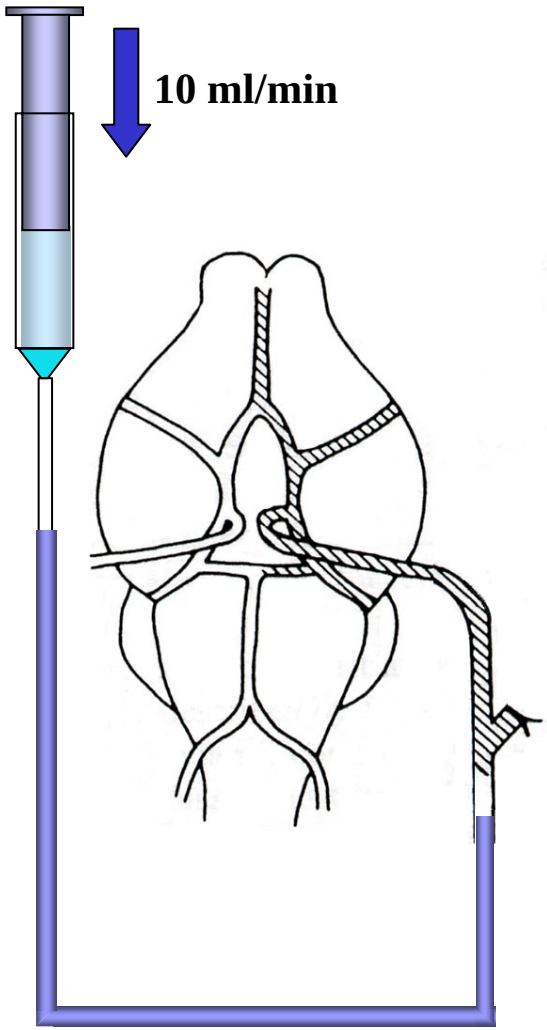
Question: **Based on knowledge about the role of efflux transporters in the blood-brain barrier, have we learned anything from these cell permeation experiments that would help predict the brain permeation of these DADLE prodrugs in animals or man?**

Answer: YES!!!!

In Situ Rat Brain Perfusion Model



- **Animals:** Male Sprague-Dawley rats (350-400 g) under anesthesia.
- **Cannula:** A polyethylene tubing (**PE-60**).
- **Artery to be infused:** the left internal carotid artery (ICA)
- **Perfusates:** Krebs/bicarbonate buffer (NaH_2PO_4, KCl, $NaHCO_3$, NaCl, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}2H_2O$, pH 7.4) containing test drug(s).
- **Perfusion process:**

  Pre-perfusion wash: 20 sec.

  Perfusion duration: 1min, 2 min, and 4 min as specified.

  Post-perfusion wash: 5–30 sec as specified.

- **Sample preparation**: After perfusion, brains were removed by decapitation and dissected on ice. The gray matters from the left cortex were weighed. Samples were either dissolved in SOLVABLE™ and then count for radioactivity or homogenized in perfusion buffer and then processed by a capillary depletion method.

BBB P_{app} Values for DADLE and Its Prodrugs in the Absence and Presence of a Pgp Inhibitor (GF120918) [a]

Reference: Chen *et al.*, *J. Pharmacol. Exptl. Therap.*, 303, 849-857, 2002; Ouyang *et al.*, *J. Pharm. Sci.*, 98, 337-348, 2009

Compound	P_{app} x 10^7 (cm/sec) [b]		Relative Increase in P_{app}
	-GF120918	+GF120918 [c]	
DADLE	0.5± 1.4	0.6± 0.14	—
AOA-DADLE	1.2± 1.0	60.5± 25.6	50
CA-DADLE	0.4± 0.7	185± 68.3	460
OMCA-DADLE	0.7± 0.6	119± 12.8	170
Quinidine	16.2± 1.1	169± 65.3	10.4

a. Apparent permeability coefficients (P_{app}) were calculated from measured K_{in} and PA based on the rat brain capillary surface area as reported (130 cm^2/g).

b. In the case of AOA-DADLE, CA-DADLE, and OMCA-DADLE, P_{app} values are based on the sum of prodrug, intermediate and DADLE presented in brain tissue.

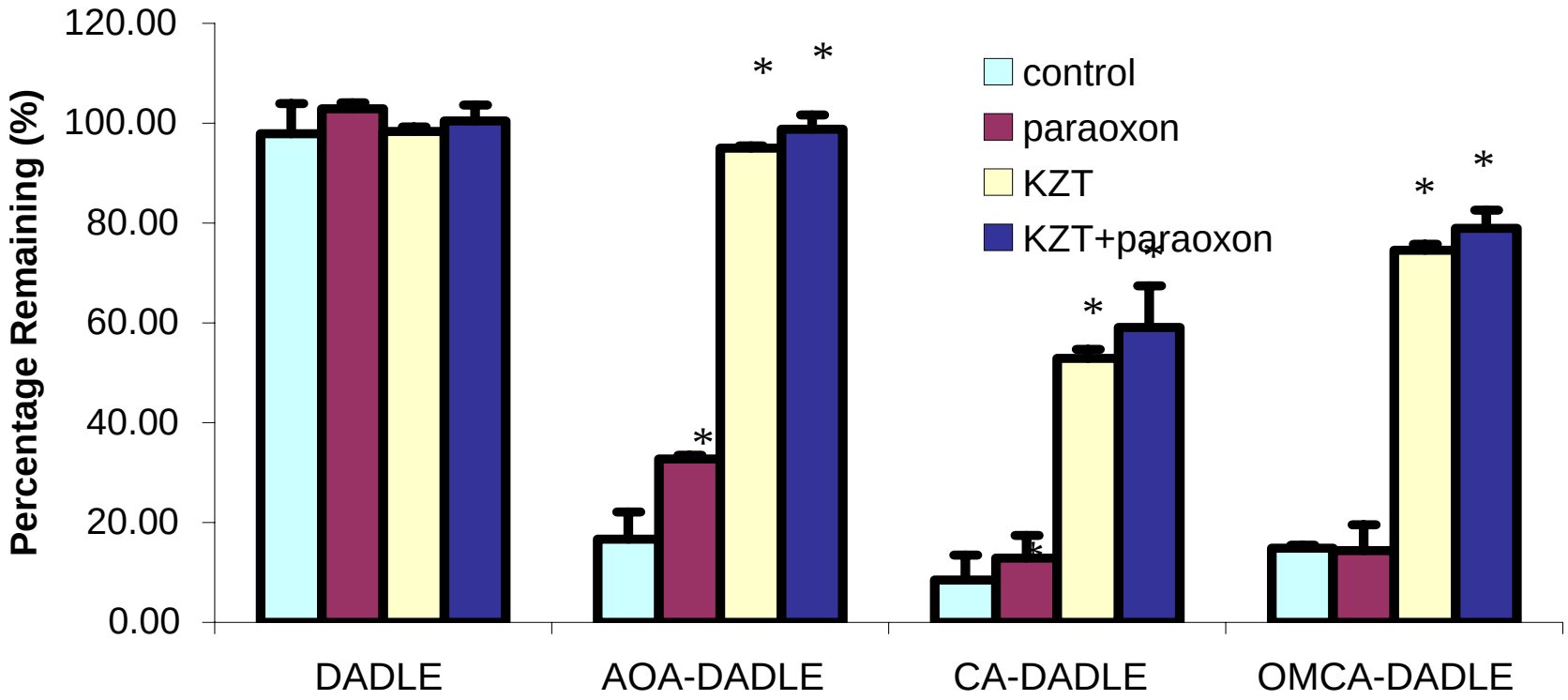
c. 10 μM GF120918 was used in these studies since this is a concentration that totally inhibited MDR1 efflux of Quinidine.

Question: **Based on knowledge about the overlap in substrate specificity between MDR1 and cytochrome P-450-3A4, have we learned anything from these cell permeation experiments that would help predict the metabolism of these DADLE prodrugs in the intestinal mucosa or liver?**

Answer: Perhaps

Stability of Prodrugs in Rat Liver Microsomes

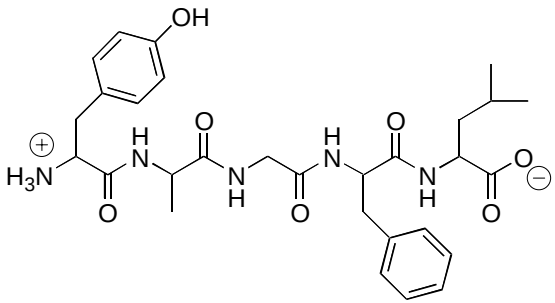
Reference: Ouyang *et al*., *J. Pharm. Sci*.., 98, 349-361, 2009



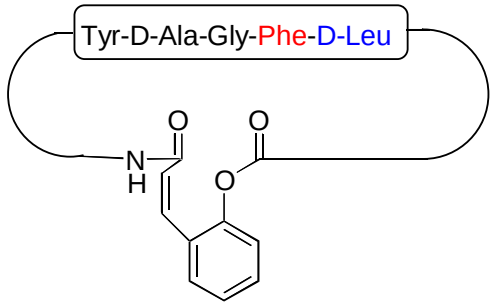
*: P < 0.05 compared to control (without inhibitor).
--- Paraoxon @ 100 μM; KZT @5 μM; prodrugs @ 2.5 μM incubated 30 min.

- Prodrugs of DADLE were rapidly metabolized in rat liver microsomes and **by hCYP3A4 (data not shown).**

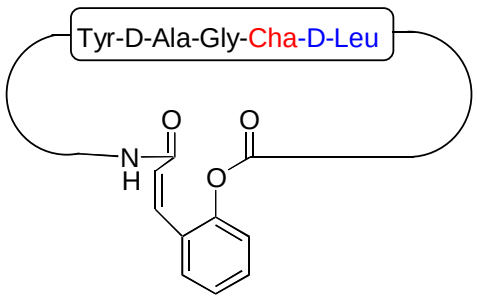
Analogs of CA-DADLE (Reference: R. Nofsinger *et. al.* unpublished data)



DADLE

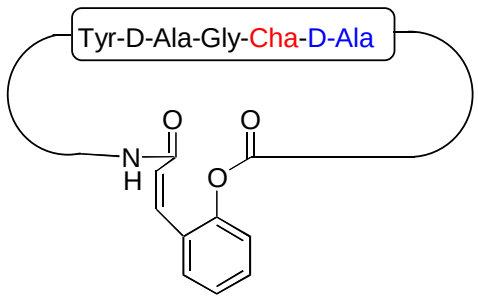


CA-DADLE



CA-DAChaDLE

(1)

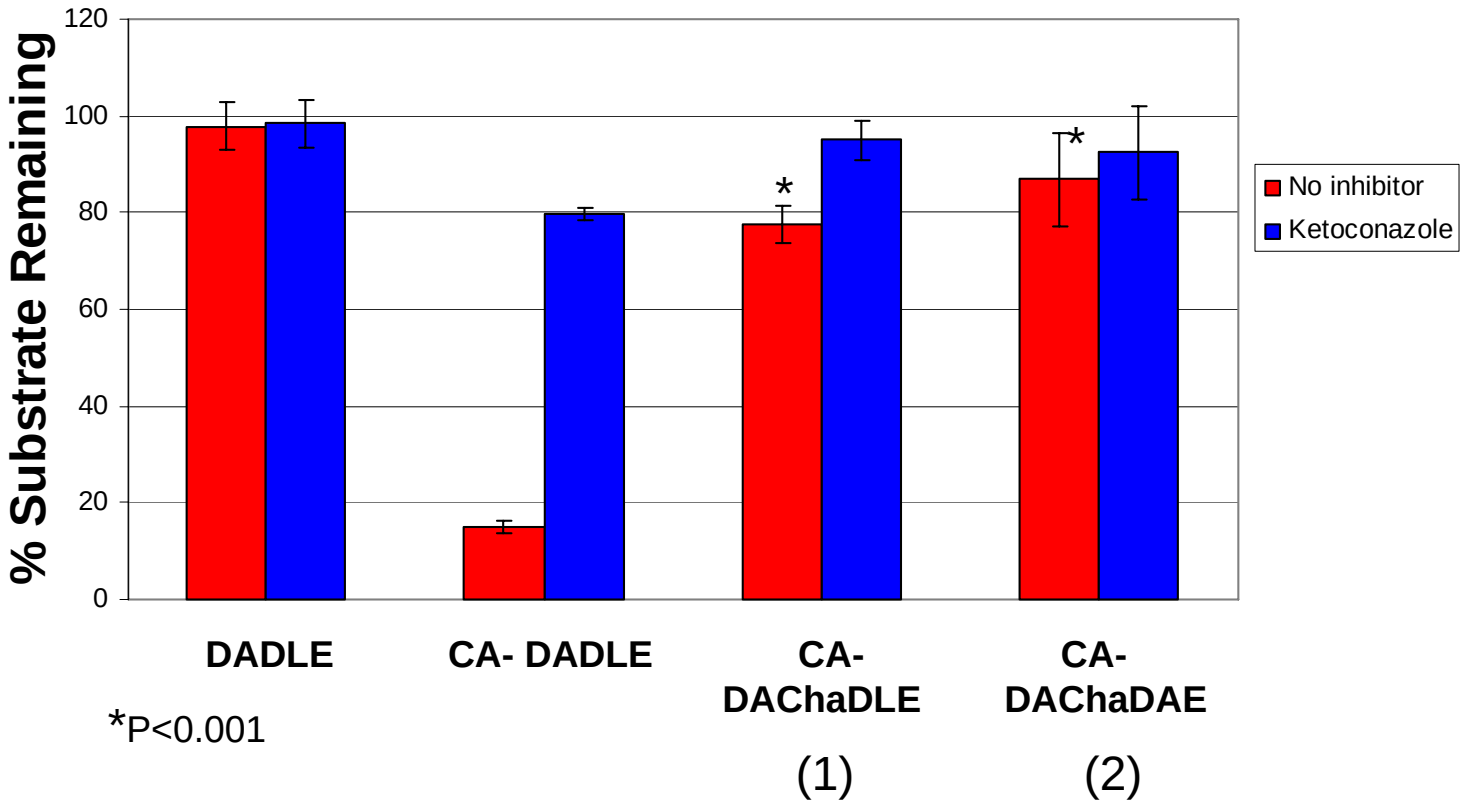


CA-DAChaDAE

(2)

Metabolic Stability of CA-DADLE and Amino acid-modified Cyclic Prodrugs of CA-DADLE in the Presence of hCYP3A4

Reference: R. Nofsinger *et. al*. unpublished data



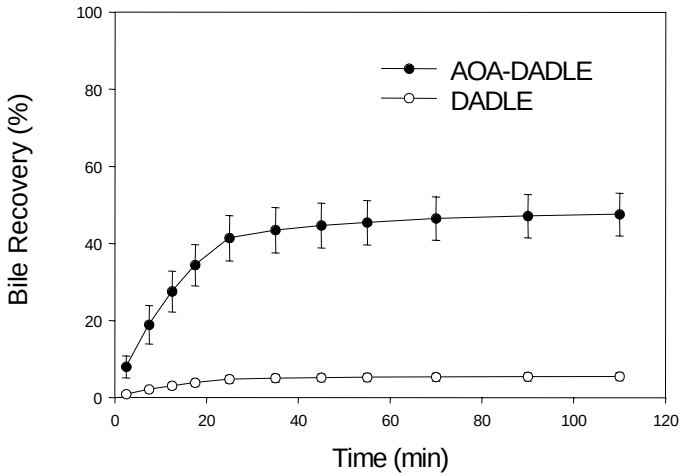
Conclusions: CA-DAChaDLE and CA-DAChaDAE are stable to metabolism by hCYP3A4, a metabolic barrier to intestinal absorption.

Question: **Based on knowledge about the role of MDR1 in the liver, have we learned anything from these cell permeation experiments that would help predict the non-metabolic clearance of these DADLE prodrugs by the liver?**

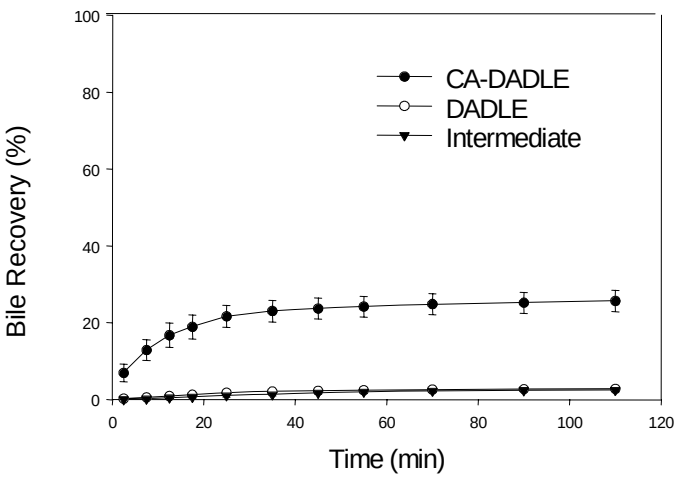
Answer: Perhaps

Time Course of Appearance of the DADLE Prodrugs and Their Metabolites in Bile after I.V. Administration of the Prodrugs to Rats (Yang *et al*., J. Pharmacol. Exptl. Therap., 303, 849-857, 2002).

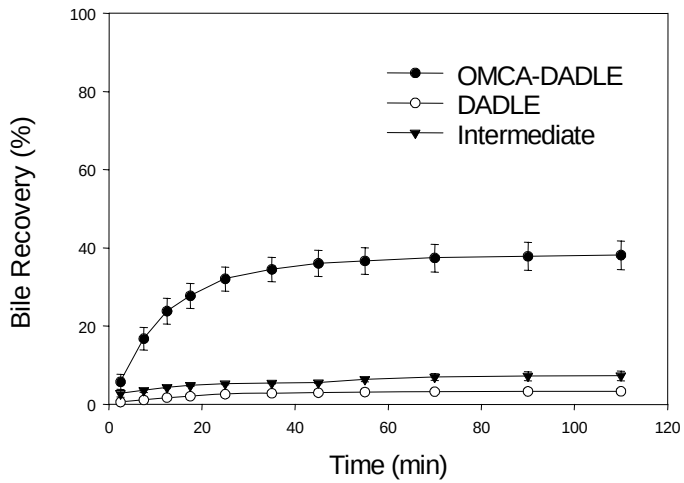
A. AOA-DADLE



B. CA-DADLE



C. OMCA-DADLE



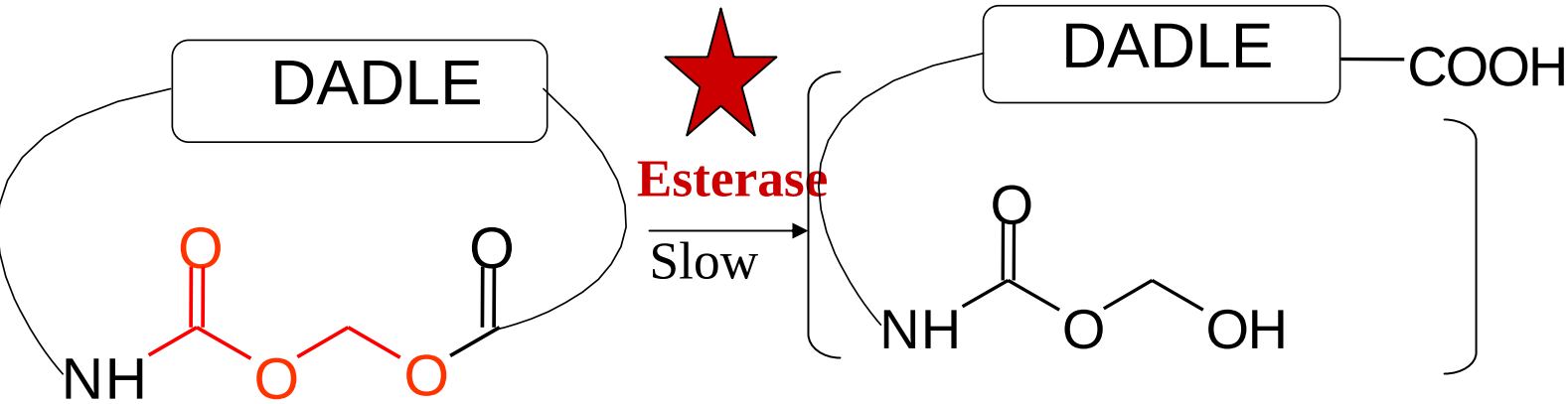
Questions

--- In what biological media (e.g. plasma, liver, brain) will the bioconversion of the cyclic prodrugs of the opioid peptides occur? **For our application, brain is the preferred biological media for bioconversion.**

--- What are the appropriate (most like human) animal models to use for the PK/toxicological evaluation of the cyclic prodrugs of the opioid peptides?

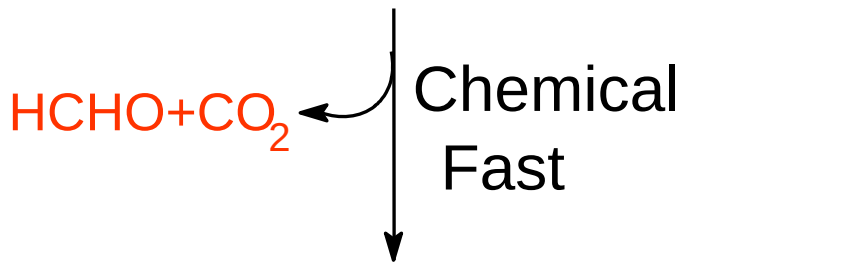
Acyloxyalkoxy (AOA)-Based Cyclic Prodrug Strategy for Improving the Cell Permeation of DADLE

Reference: A. Bak *et al.*, *Pharm. Res.*, 16, 24-29, 1999



AOA-DADLE

Hydrophobic
(Transcellular Permeant)

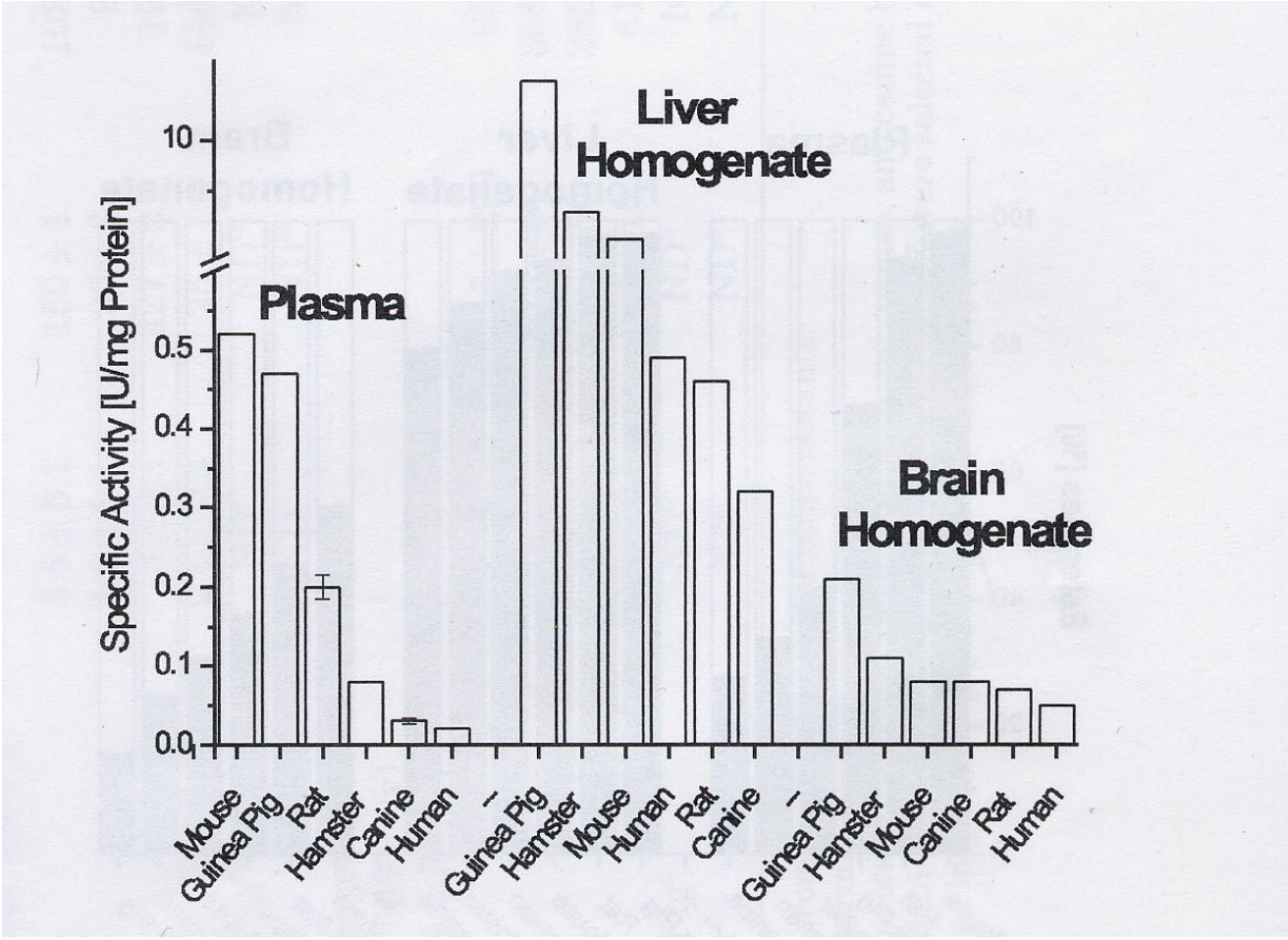


Hydrophilic
(Paracellular Permeant)

Predicted Permeabilities:
AOA-DADLE>>>>DADLE

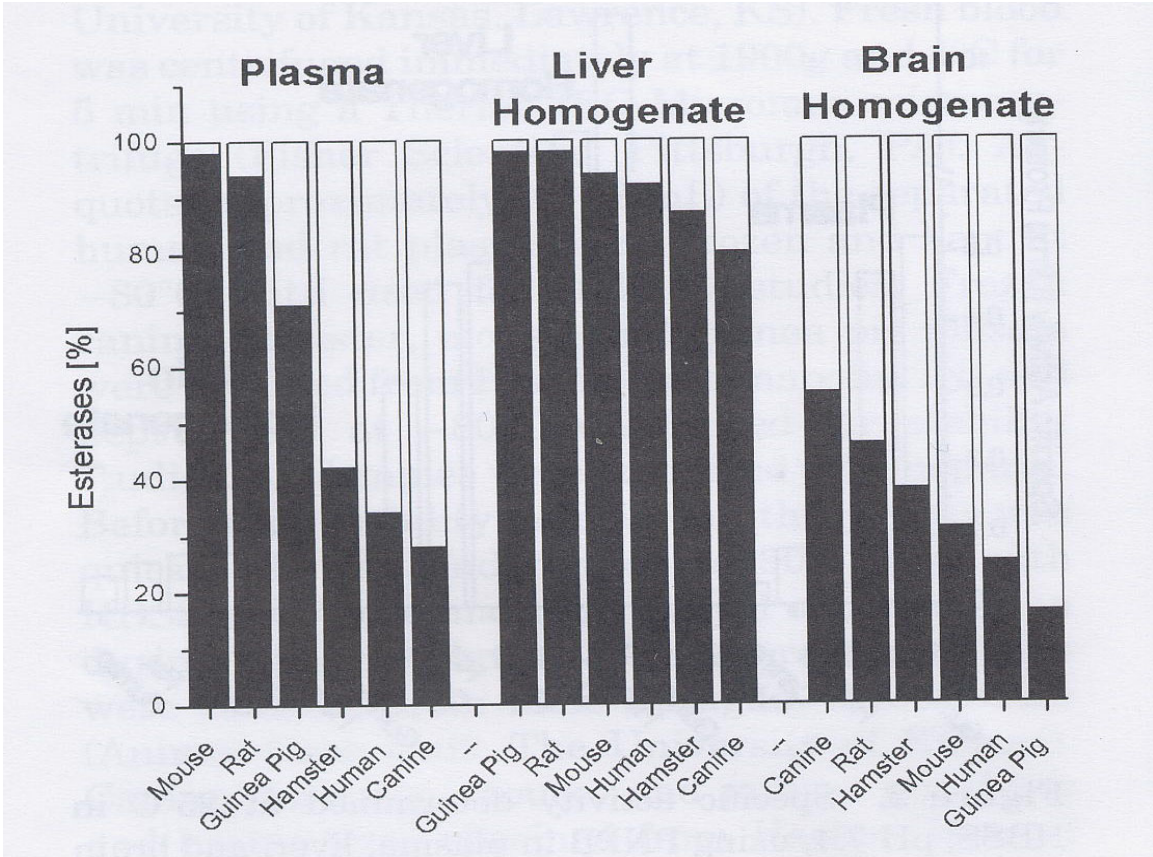
Specific Activity of Total Esterase in Plasma, Liver and Brain from Various Animal Species

Reference: Liederer and Borchardt, *J. Pharm. Sci.* , 94, 2198-2006, 2005



Relative Amounts of Esterase B and Esterase A/C in Plasma, Liver and Brain from Various Animal Species

Reference: Liederer and Borchardt, *J. Pharm. Sci.* , 94, 2198-2006, 2005



Esterase B, dark shading; Esterase A/C, no shading

Apparent Half-Lives ($t_{1/2}$) of OMCA-[D-Ala^2,Leu^5]-Enk in Plasma, and Brain Homogenates from Different Animal Species

References: Liederer and Borchardt, *J. Pharm. Sci.* , 94, 2198-2006, 2005; Liederer et al., *J. Med. Chem*., 49, 1261-1270, 2006

	$T_{1/2}$ mins, mean ±SD, n=3	
Tissue/Medium	OMCA-[D-Ala^2,Leu^5]-Enk	B/P Ratio
Plasma		
Human	**231±5.6**	**5.0**
Rat	< 2	0.14
Mouse	2 ±0.5	0.09
Canine	**148±37**	**7.05**
Guinea Pig	**23±1**	**1.77**
Brain		
Human	**46±2**	
Rat	14±4	
Mouse	22±2	
Canine	**21±3**	
Guinea Pig	**13±0.4**	

Conclusions from Rat Studies

- The substrate activity of these cyclic prodrugs for efflux transporters limit their oral absorption and their permeation across the BBB, as well as facilitate their biliary clearance in rats.
- The substrate activities of these cyclic prodrugs for rat cytochrome P-450s facilitate their metabolic clearance in the intestinal mucosa and the liver.
- Rates of prodrug bioconversion of these cyclic prodrugs are tissue, species, and prodrug specific. From this perspective, **guinea pig is a preferred species over rat for PK and toxicity studies.**

Guinea Pig Studies

Brain Uptake of Cyclic Prodrugs

Reference: Liederer *et al.*, *J. Pharm. Sci.*, 94, 2676-2687, 2005

Species	Compound	Brain Uptake (ng/g tissue)	
		Prodrug	Linear Peptide**
Rat*	OMCA-DADLE	3.30 ± 0.33	1.34 ± 1.50
Guinea Pig	OMCA-DADLE	263.6 ± 114.8	11.5 ± 2.6

*Yang et al. *J. Pharmacol. Exptl. Therp., 303, 840-848, 2002*

** DADLE or D-Ala-Enk

Bile Recovery of Cyclic Prodrugs

Reference: Liederer *et al.*, *J. Pharm. Sci.*, 94, 2676-2687, 2005

		Bile Recovery of Dose (%, mean±SE)	
Species	**Compound**	**Prodrug**	**Linear Peptide****
Rat*	OMCA-DADLE	38.1±2.1	3.3 ±0.4
Guinea Pig	OMCA-DADLE	3.3±0.4	0.11±0.01

*Yang et al. *J. Pharmacol. Exptl. Therp., 303, 840-848, 2002*

** DADLE or D-Ala-Enk

Conclusions from Guinea Pig Studies

- These cyclic prodrugs exhibit excellent permeation across guinea pig BBB.

- These cyclic prodrugs are substrates for guinea pig cytochrome P-450s facilitating their metabolic clearance in the intestinal mucosa and the liver.

- These cyclic prodrugs do not undergo significant biliary clearance in guinea pigs.

Question

Will humans handle these prodrugs like rats or like guinea pigs??????

Conclusions-The Good News

- ✓ Cyclic prodrugs of opioid peptides have more favorable physicochemical properties (e.g., hydrophobicity, low hydrogen bonding potential, no charge) for cell permeation.
- ✓ Cyclic prodrugs of opioid peptides exhibit good “intrinsic” cell permeation characteristics.
- ✓ These cyclic prodrugs are all substrates for esterases that catalyze their bioconversion to the opioid peptide. Some cyclic prodrugs are bioconverted more rapidly in **BRAIN THAN BLOOD**. **However, this phenomena is species-dependent!!!!!!!**

Conclusions-The Bad News

- ✓ Cyclic prodrugs of opioid peptides are substrates for efflux transporters that limit their permeation across the intestinal mucosa and the BBB. **However, this phenomena is species-dependent!!!!!!!**
- ✓ Cyclic prodrugs of opioid peptides are rapidly cleared by the liver into the bile. **However, this phenomena is species-dependent!!!!!!!**
- ✓ Cyclic prodrugs of opioid peptides are substrates for cytochrome P-450 enzymes which contribute to their high clearance.
- ✓ Some cyclic prodrugs are bioconverted more rapidly in **BLOOD THAN BRAIN**. **However, this phenomena is species-dependent!!!!!!!**
- ✓ CA and OMCA-based cyclic prodrugs generate “stable” intermediates that slowly convert chemically *in vivo* to the opioid peptide.