

# Chemistry and Biochemistry of COMT Inhibitors

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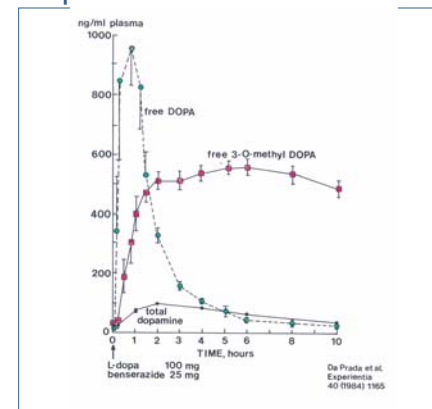
# Inhibition of Catechol O Methyltransferase Enzyme (COMT) – Why?

- Dopamine replacement has been the major therapy for the treatment of Parkinson's disease over 40 years
- Dopamine precursor treatment
  - L-dopa or by modifying L-dopa metabolism with
    - Aromatic amino acid decarboxylase (Dopa decarboxylase = DDC) inhibitors (carbidopa, benserazide)
    - Catechol O-methyltransferase (COMT) inhibitors (entacapone, tolcapone)
    - L-dopa prodrugs (melevedopa)
- Dopamine agonists
  - directly acting dopamine mimics
    - D1/D2/D3 receptor (cabergoline, pramipexole, ropinirole, rotigotine)
- Dopamine metabolism inhibitors
  - monoamine oxidase inhibition (+ central COMT inhibitors)
    - MAO-B selective inhibitors (selegiline, rasagiline)
- Dopamine reuptake inhibitors
  - Selective compounds not on the market

# Treatment of Parkinson's Disease with L-dopa – Why?

- L-dopa has remained the most effective drug for treating of Parkinson's disease = Gold standard
- L-dopa has been used over 40 years (1967)
- Bioavailability of L-dopa is poor
  - Only 1% of orally administered L-dopa reaches the brain
- Bioavailability of L-dopa improved by co-treatment with dopa decarboxylase inhibitors benserazide or carbidopa
  - Madopar (L-dopa + benserazide)
  - Sinemet (L-dopa + carbidopa)
  - Bioavailability 10 %?
- DDC treatment shifts metabolism to O-methylation by COMT
  - 90 % of L-dopa spared from decarboxylation is O-methylated

# Peripheral Metabolism of Levodopa in the presence of AADC inhibition



## Catechol O methyltransferase Enzyme (COMT; EC 2.1.1.6)

- The general function of COMT is the elimination of biological, active or toxic catechols and their hydroxylated metabolites
- The enzyme-catalyzed O-methylation of catecholamines was first described by Axelrod and co-workers in the late 1950s – the responsible enzyme was called catechol O-methyltransferase (COMT)
- COMT is currently known to be present in two isoforms
  - a cellular soluble COMT (Sol-COMT)
  - a membrane-bound COMT (MB-COMT)
- In mammals, COMT enzyme is distributed throughout various organs. The highest activities are found in liver and kidney

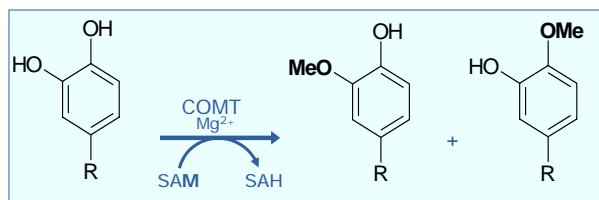
## COMT Research

### COMT Gene Expression

#### ONE GENE – TWO PROTEINS - POLYMORPHISM

- One gene codes for Sol- and MB-COMT
- Located on chromosome 22 band q11.2
- Functionally polymorphic (Val<sup>108/158</sup>Met):
  - low (COMT<sup>L</sup>) (met/met)
  - medium (COMT<sup>LH</sup>) (met/val)
  - high (COMT<sup>H</sup>) (val/val)
- Polymorphism not in the active site
  - does not seem to affect COMT inhibition

## Reaction Catalyzed by COMT



- COMT has a broad substrate specificity: Catecholamines, Catecholestrogens, Flavonoids etc. and L-dopa
- COMT is stringent for S-Adenosyl-L-Methionine (SAM) as the methyl donor
- COMT needs a divalent metal cation (Mg<sup>2+</sup>) for activity

## Distribution of COMT Activity in Rat Tissues

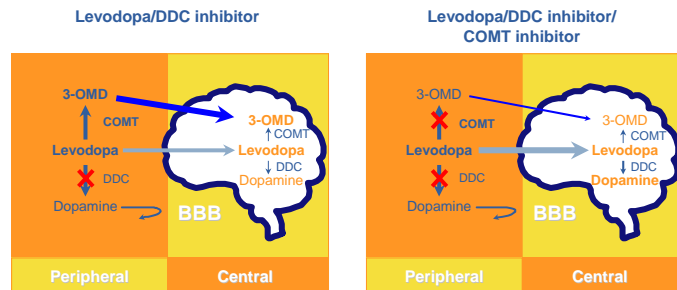
Tissue	COMT activity* (pmol/min/mg protein)
Liver	4580
Kidney	1594
-----	
GI-tract	
• stomach	283
• duodenum	660
• ileum	544
• jejunum	508
• colon	476
-----	
Thymus	599
Spleen	321
Aorta	284
Lungs	180
Brain	119
Erythrocyte	34
Muscle	21

\*DHBac as the substrate

unpublished data

## Reducing the Peripheral Metabolism of Levodopa

Addition of COMT inhibitor decreases conversion of levodopa to 3-OMD in the periphery



Abbreviations: DDC = dopa decarboxylase BBB = blood-brain barrier

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## Properties of Earlier COMT Inhibitors

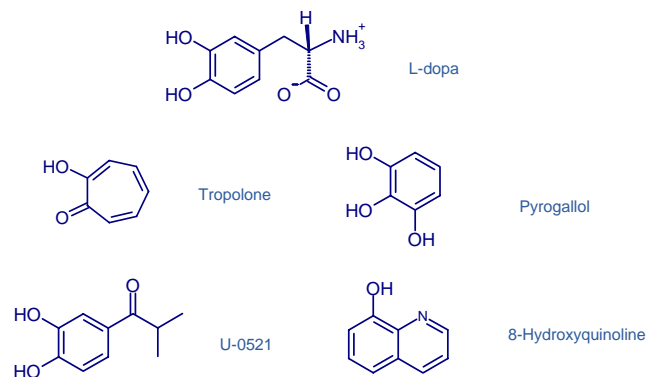
Type of Inhibitor (Astra 1960's)	Efficacy ( $\mu\text{M}$ )	
	<i>in vitro</i>	<i>in vivo</i>
Catechols	2 – 32	poor
Flavonoids	1 – 10	poor
Pyrogallols	1 – 50	poor
Quinolines	1 – 10	poor
Tropolones	30 – 50	poor
(Upjohn 1960 - 70's)		
Benzoic acids	10 – 50	weak
Catechol ketones	1 – 10	weak

Poor or weak efficacy due to toxicity and ADME properties

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## Chemical Structures of Old COMT Inhibitors



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## Bisubstrate COMT Inhibitors

- Combine catechol + SAM/SAH
- Described first at 1981 by Yale researchers as Multisubstrate Adducts<sup>(1)</sup>  
→  $K_i$  800  $\mu\text{M}$ ! poor inhibitors
- Soon after at 1983 by Wellcome Research Laboratories as Bridget Catechol-Homocysteine Derivatives<sup>(2)</sup>  
→ poor inhibition c. 15% at 100  $\mu\text{M}$
- Finally at 2003 by Roche Nitrocatechol + SAM as Bisubstrate inhibitors<sup>(3)</sup>  
→  $K_i$  = 9 nM

<sup>1)</sup> Anderson et al. *J Med Chem* 1981; 24: 1271.

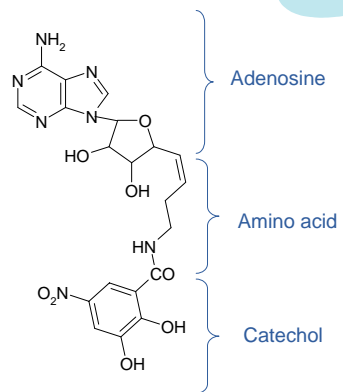
<sup>2)</sup> Lever et al. *J Pharm Sci* 1984; 73: 1241

<sup>3)</sup> Lerner et al. *Helv Chim Acta* 2003; 86: 1045

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### Bisubstrate COMT Inhibitors\*



\*Lerner *et al. Helv Chim Acta* 2003; 86: 1045

### Selectivity of Old COMT-Inhibitors

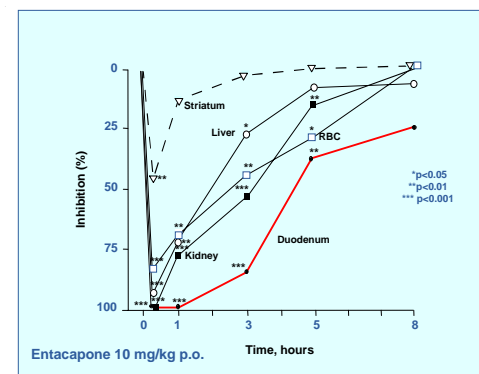
Compound	Enzyme: IC <sub>50</sub> -value (µM)		
	COMT	Tyrosine hydroxylase	Dopamine-β-hydroxylase
U-0521	6	24	>50
Propylgallate	2	15	>50
Tropolone	35	> 50	5

### Selectivity of a Nitrocatechol Structured COMT Inhibitor Entacapone

Enzyme	IC <sub>50</sub> -value
<b>COMT</b>	<b>0.010 µM</b>
Tyrosine hydroxylase	48 µM
Dopamine-β-hydroxylase	>50 µM
Dopa decarboxylase	>50 µM
MAO-A and -B	>50 µM
Other catecholamine metabolising enzymes and methyltransferases	>50 µM

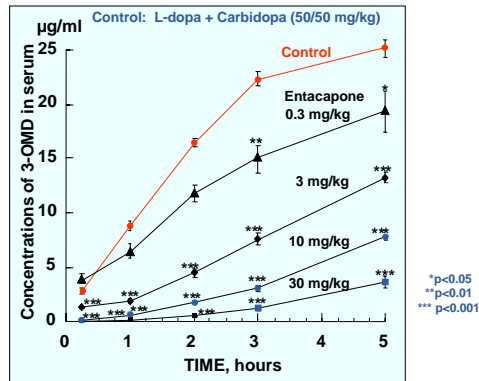
Nissinen *et al. Naunyn Schmiedebergs Arch Pharmacol* 1992; 346: 262-6.

### Inhibition of COMT Activity in Rat Tissues by Entacapone



Nissinen *et al. Naunyn Schmiedebergs Arch Pharmacol* 1992; 346: 262-6.

## Dose- and Time-Related Decrease in 3-OMD Concentrations in Rat by Entacapone



Nissinen et al. *Naunyn Schmiedebergs Arch Pharmacol* 1992; 346: 262-6.

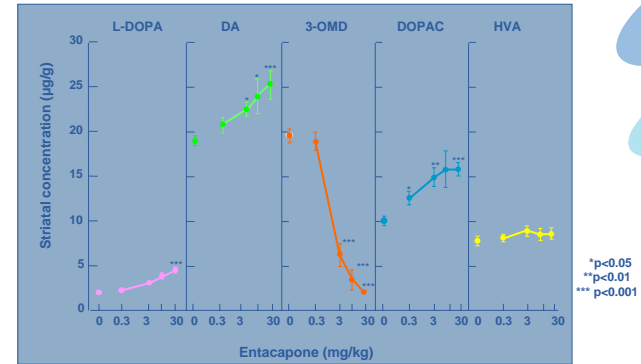
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## Effect of Entacapone on Levodopa and its Metabolites in Rat Striatum



Nissinen et al. *Naunyn Schmiedebergs Arch Pharmacol* 1992; 346: 262-6.

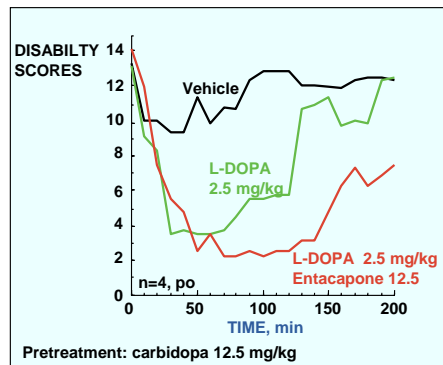
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## Effect of Entacapone on Disability Scores in MPTP-Treated Marmosets



Smith et al. *Mov Dis* 1997; 12: 435

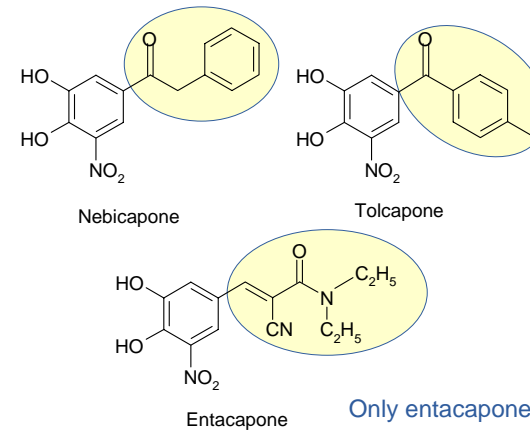
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## CLINICALLY TESTED NITROCATÉCHOL COMT INHIBITORS



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## CLINICALLY TESTED/USED COMT INHIBITORS <sup>21</sup>

### Entacapone:

- Used alone Comtess®/Comtan® with L-dopa + carbidopa
- and in combination form: Stalevo® (L-dopa+carbidopa+entacapone)

- Is safe and effective in clinical use
  - 1.300.000 patient years

### Tolcapone:

- Can be used as a COMT inhibitor but safety!
  - requires liver enzyme monitoring

### Nebicapone

- Liver enzymes elevated in Phase II
  - development discontinued (Spring 2008)



## Nitrocatechols as COMT inhibitors <sup>22</sup>

- Inhibition of COMT activity in vitro
  - Adrenaline as the substrate

	IC <sub>50</sub> -values (nMol/l)		Ki (nMol/l)
	Brain COMT	Liver COMT	Pure COMT
Nebicapone	3.7 (nd)	696 (nd)	0.7
Entacapone	12.8 (10*)	2320 (160*)	1.6
Tolcapone	2.2 (24*)	927 (254*)	1.9

(\*IC<sub>50</sub>- and Ki-values determined at Orion)

Conclusion: All are potent COMT inhibitors in vitro



## Nitrocatechols as COMT Inhibitors <sup>23</sup>

Inhibition of COMT activity ex vivo after 30 mg/kg p.o. to rat

### Liver Time Course of COMT Inhibition (%)

	0.5 h	1 h	3 h	6 h	9 h
Nebicapone	99	97	96	76	70
Entacapone	98	96	86	74	25
Tolcapone	100	99	98	94	67

Learmonth et al. *J Med Chem* 2002; 45: 685



## Nitrocatechols as COMT Inhibitors <sup>24</sup>

Inhibition of COMT activity ex vivo after various doses of drugs

Drug	ED <sub>50</sub> (mg/kg, p.o. at 1 h)	
	Liver	Brain
Nebicapone	0.7	5.3
Entacapone	1.9	> 30
Tolcapone	0.7	1.6

Learmonth et al. *J Med Chem* 2002; 45: 685



## Nebicapone as a COMT Inhibitor

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Prediction of tissue COMT inhibition by RBC COMT inhibition?

ED <sub>50</sub> (mg/kg, p.o. at 1 h)		
	Liver	RBC
Nebicapone	1.9	1.9

Soares-da-Silva *et al. Pharmacol Toxicol*; 2003; 92: 272

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## Nebicapone in Clinical Studies: Phase I

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**Single dose study** (50, 100, 200 and 400 mg of NbC + Madopar 125) n = 18

### Results:

- L-dopa AUC increased 39% - 80% (no effect on Cmax; t<sub>1/2</sub> = 60%)
- 3-OMD AUC decreased 38% - 62%
- RBC COMT Inhibition 57% - 84% (Maximal inhibition at 200 mg)

### Conclusions:

- L-dopa/3-OMD:

Nebicapone is more potent than Entacapone (50 mg = 200 mg EC)  
Less potent than Tolcapone (200 mg = 150 mg TC)

### Safety:

Nebicapone is safe and well tolerated COMT inhibitor

Silveira *et al. Eur J Clin Pharmacol* 2003; 59: 603

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## Nebicapone in Clinical Studies: Phase I

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Single dose study (50, 100, 200 and 400 mg of BIA + Madopar 125) n = 18

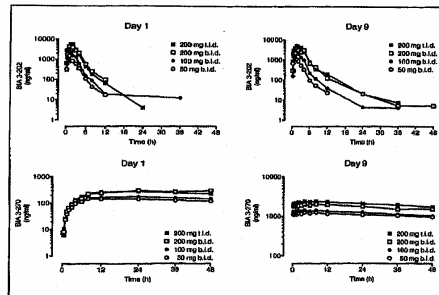


Figure 1. Mean plasma concentration-time profiles of BIA 3-202 and BIA 3-270 following single oral doses (day 1) and repeated doses (day 9) of 50 mg bid, 100 mg bid, 200 mg bid, and 200 mg tid. Symbols represent means of six to seven determinations per group.

Almeida & Soares-da-Silva, *J Clin Pharmacol* 2003; 43: 1350

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## Nebicapone in Clinical Studies: Phase I

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Multiple-dose study (50, 100, 200 bid and 200 mg tid of Nebicapone)  
n = 6 - 8/group; treatment duration 9 days

### Results of Pharmacokinetics:

- metabolite BIA 3-270 (3-O-Me) t<sub>1/2</sub> = 70 - 90 h

### Tolerability

- 61% reported mild typical COMT-I related AEs  
headache, dizziness, nausea etc
- side-effects not dose related

**Conclusions:** Nebicapone is well tolerated effective COMT inhibitor

Almeida & Soares-da-Silva, *J Clin Pharmacol* 2003; 43: 1350

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## Nebicapone in Clinical Studies: Phase II

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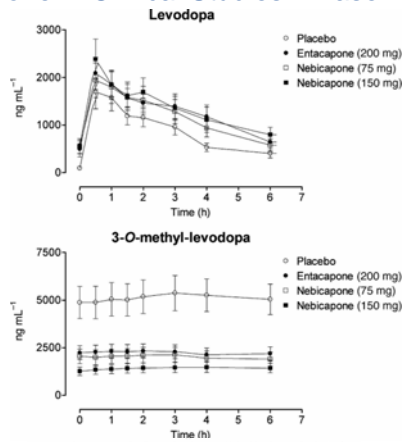


FIGURE 2. Plasma concentration-time profiles (mean  $\pm$  SEM) of levodopa and 3-O-MD after administration of placebo, 75 and 150 mg nebicapone, and 200 mg entacapone concomitantly with Sinemet 25/100.

Ferreira *et al. Clin Neuropharmacol* 2008; 31: 2

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## The Effects of Tolcapone and Entacapone on the response duration (ON-time) of single morning dose of L-dopa

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### SUMMARY

	tolcapone (100-200mg)	entacapone (200mg)
L-dopa T <sub>1/2</sub>	+79% (+46min)	+27-39% (+20-24min)
L-dopa AUC	+58%	+23-35%
ON-time	+64-69% (+54-60min)	+21-27% (+21-24min)
	Nb +22 - 45 min	

Tolcapone: Limousin *et al. Clin Neuropharmacol* 1995; 18: 258-265. Roberts *et al. Neurology* 1994; 44: 2685-2688  
 Entacapone: Merello *et al. JNNP* 1994; 57: 186-189. Ruottinen and Rinne. *Clin Neuropharmacol* 1996; 19: 283-296  
 Ruottinen and Rinne. *JNNP* 1996; 60: 36-40. Heikkinen *et al. Clin Neuropharmacol* 2001; 24: 150-157

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## Nebicapone as a COMT Inhibitor

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- *In Vitro* equal with Entacapone and Tolcapone
- *In Vivo* in Rats closer to Tolcapone
- In Humans so far closer to TOLCAPONE
- **LIVER SAFETY?**  
 →Nebicapone is like tolcapone a potent uncoupler!

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## CLINICALLY TESTED/USED COMT INHIBITORS

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Why is entacapone safe?

Chemistry and biochemistry of entacapone predicted safety!

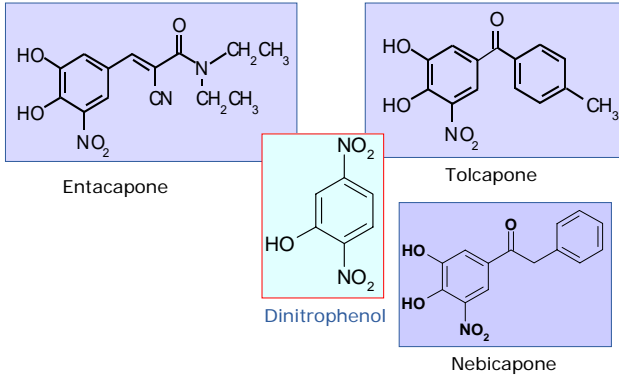
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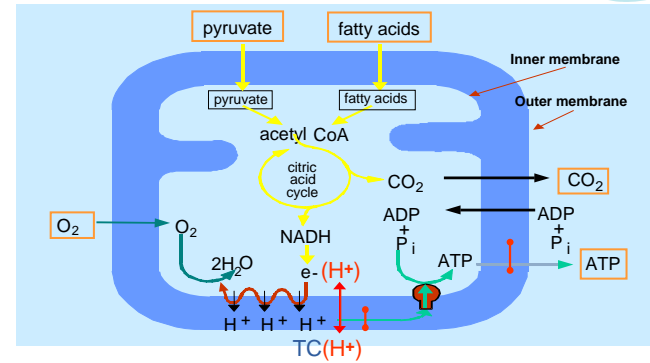
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### Chemical Structures of Entacapone, Nebicapone and Tolcapone vs. Dinitrophenol



### Mitochondrial Energy Metabolism



Protonophoric agents like TC(H<sup>+</sup>) can interfere with the transport of protons through mitochondrial membrane



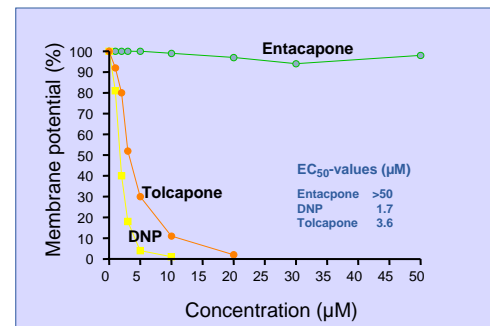
### Tolcapone and fulminant hepatitis

*Frédéric Assal, Lauren Spahr, Antoine Hadengue, Laura Rubbici-Brandt, Pierre R Burkhard*

THE LANCET • Vol 352 • September 19, 1998

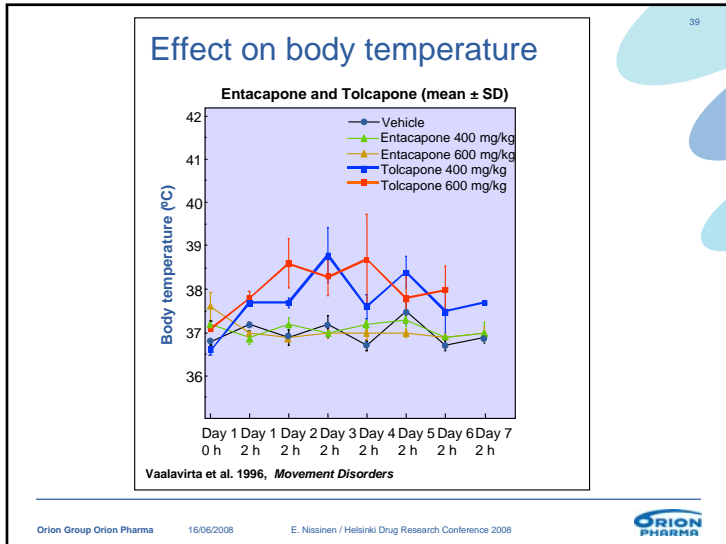
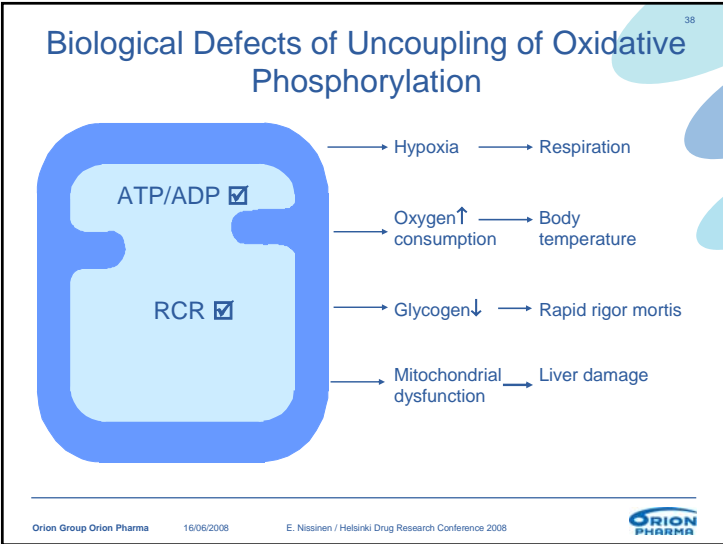
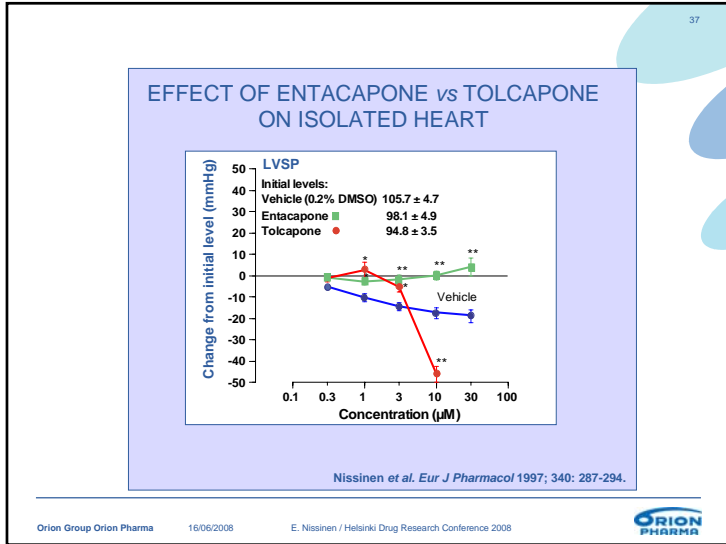


### Effect of Entacapone, Tolcapone and DNP on Mitochondrial Membrane Potential



Haasio et al. Eur J Pharmacol 2002; 453: 21-26





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### Comparative toxicity studies in rat with entacapone and tolcapone

#### Role of uncoupling of oxidative phosphorylation

Compound and dose level (mg/kg/day)	Mitochondrial ATP/ADP	Body temperature	Liver enzymes	Relative liver weight	Liver, histological findings	Onset of rigor mortis
Dinitrophenol 20	↓	↑	↑	↑	centrolobular hypertrophy	<10 min
Entacapone 300	—	—	—	—	—	—
Entacapone 500	—	—	—	—	—	—
Tolcapone 300	(↓)	↑	↑	↑	—	<10 min
Tolcapone 500	↓	↑	↑	↑	centrolobular hypertrophy hydropic vacuolation	<10 min

→ no effect Haasio et al J Neural Transm 2002; 109: 1391-1401

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## Comparative toxicity studies with entacapone and tolcapone

### Mitochondrial findings in the liver and skeletal muscle

Organ /Finding Dose mg/kg/day	DNP	Entacapone	Tolcapone	
	20	500	300	500
Mitochondrial swelling	+	-	-	+
Disrupted mitochondria	+	-	-	+
Reduced matrix density	+	-	-	+
Deformed cristae	+	-	+	+

Haasio et al. *Exp Toxic Pathol* 2002; 54: 9-14

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## Pathology of COMT Knockout Mice

### COMT-deficient mice

- no major clinical symptoms
- no relevant histopathological findings
- no changes in clinical chemistry
- no changes in hematology
- no changes in behaviour

⇒ reduction of catecholestrogen O-methylation is not toxic  
 ⇒ reduction of COMT activity does not cause liver problems

Haasio et al. *J Appl Toxicol* 2003; 23: 213-219

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## SAFETY CONCLUSIONS

- COMT inhibition is not toxic: COMT k/o mice and chronic toxicity studies + Entacapone clinical use > 1.3 million patient years
- Entacapone does not have hepatotoxic potential
- Uncouplers are hepatotoxic: tolcapone and nebicapone induce elevation of liver enzyme activity (ASAT/ALAT)
- Tolcapone causes hyperthermia and liver damage in rats
- Liver toxicity of nebicapone and tolcapone may be explained by:
  - uncoupling of oxidative phosphorylation
  - nitro reduced metabolites
  - long-lived O-methylated metabolite
  - effect on biliary functions (transporters)

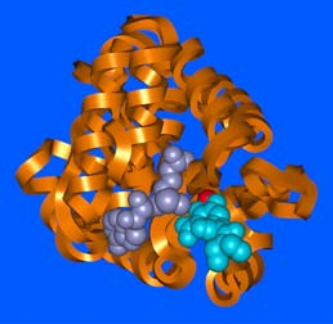
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## Is There Need/Room for a New COMT Inhibitor? – Yes!

- Tolcapone is clinically more effective than Entacapone
  - But the safety!
- Bimodal\* administration of Entacapone seems to increase L- dopa bioavailability
  - COMT inhibitor with longer duration of action?



Vidgren et al. *Nature* 1994; 368

\* Bet et al. *Eur J Neurol* 2008;15:268

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