Molecular and biochemical resolution of pyridoxine-dependent epilepsy

alpha-aminoadipic semialdehyde
as diagnostic marker

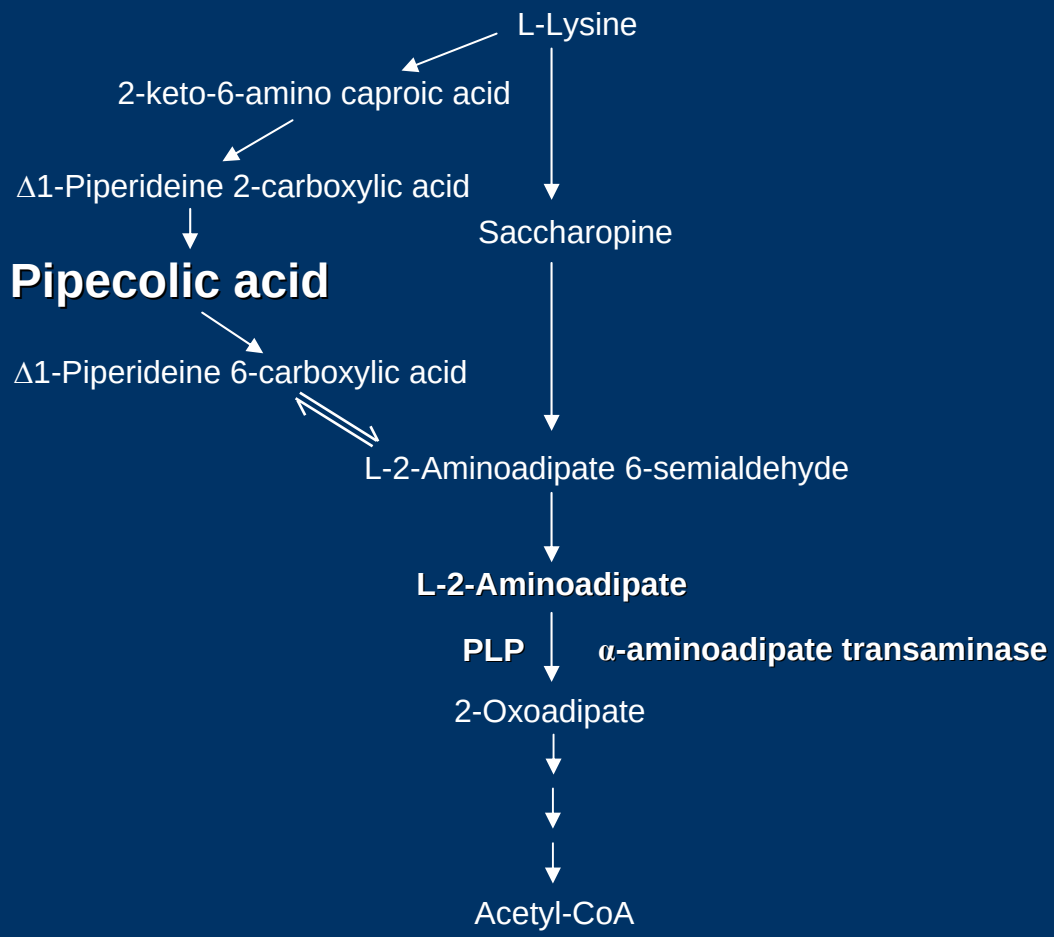
Pyridoxine-dependent seizures

- First described in 1954
- Neonatal seizures beginning in the first few days of life
- Cessation of seizures after administration of 50-100mg pyridoxine
  Continued seizure control on 15 mg/kg/d pyridoxine
  Seizure recurrence if pyridoxine is withdrawn
- Autosomal recessive
- No gene has been identified; favoured hypothesis was defect in glutamate decarboxylase (GAD)
- maps to 5q31.2 – q31.3 (Cormier-Daire *et al.*, Am J Hum Genet, 2000)

Pyridoxine-dependent seizures

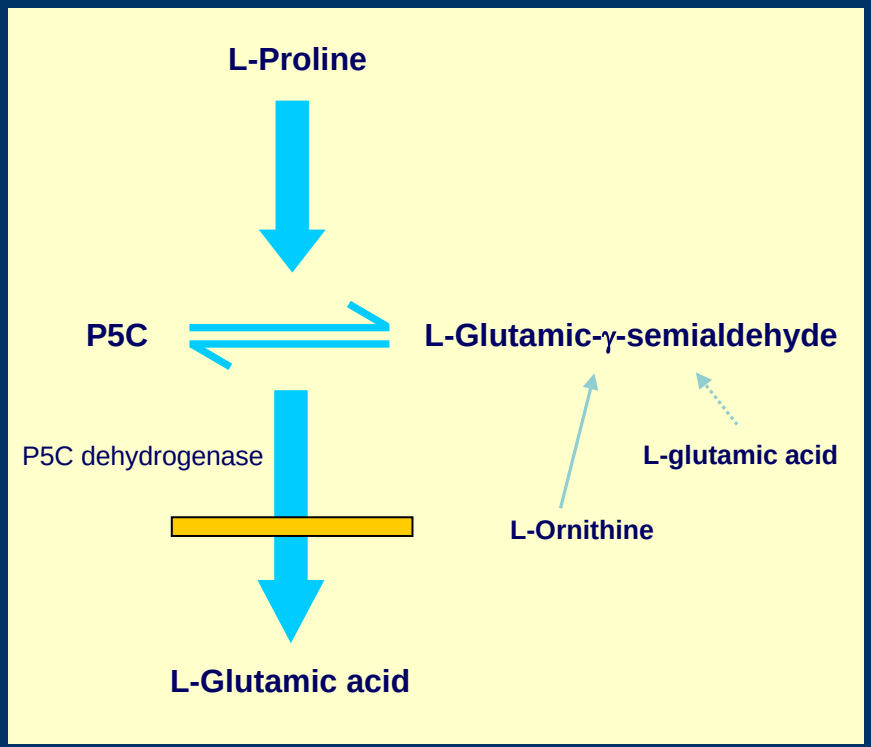
- Elevated concentrations of pipecolic acid in plasma and CSF

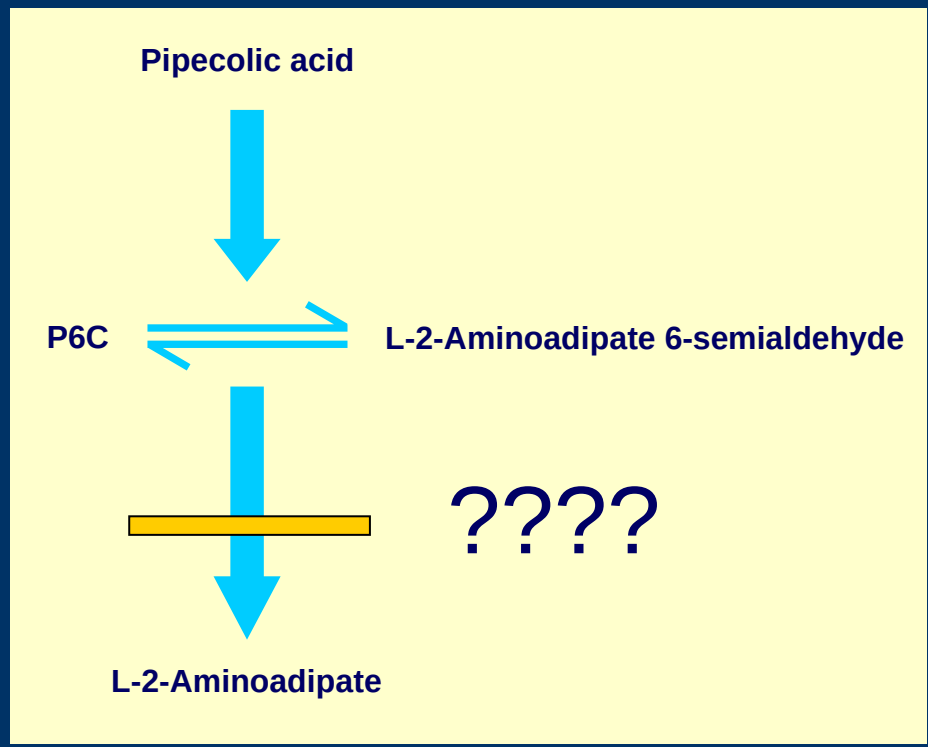
- Defect in PLP-dependent step in lysine degradation pathway ?



Pyridoxine-dependent seizures

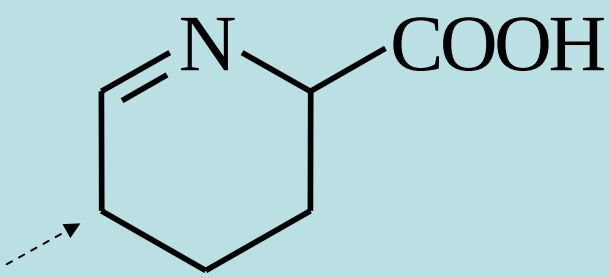
- similar scenario to that of **hyperprolinaemia type II** ?



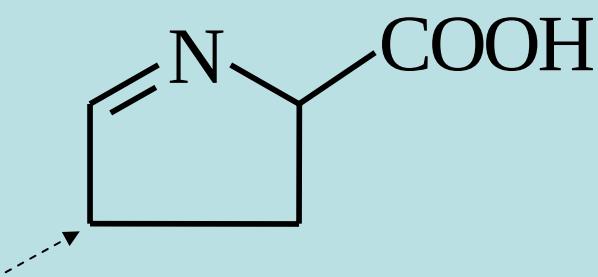


P5C reacts with PLP to inactivate it

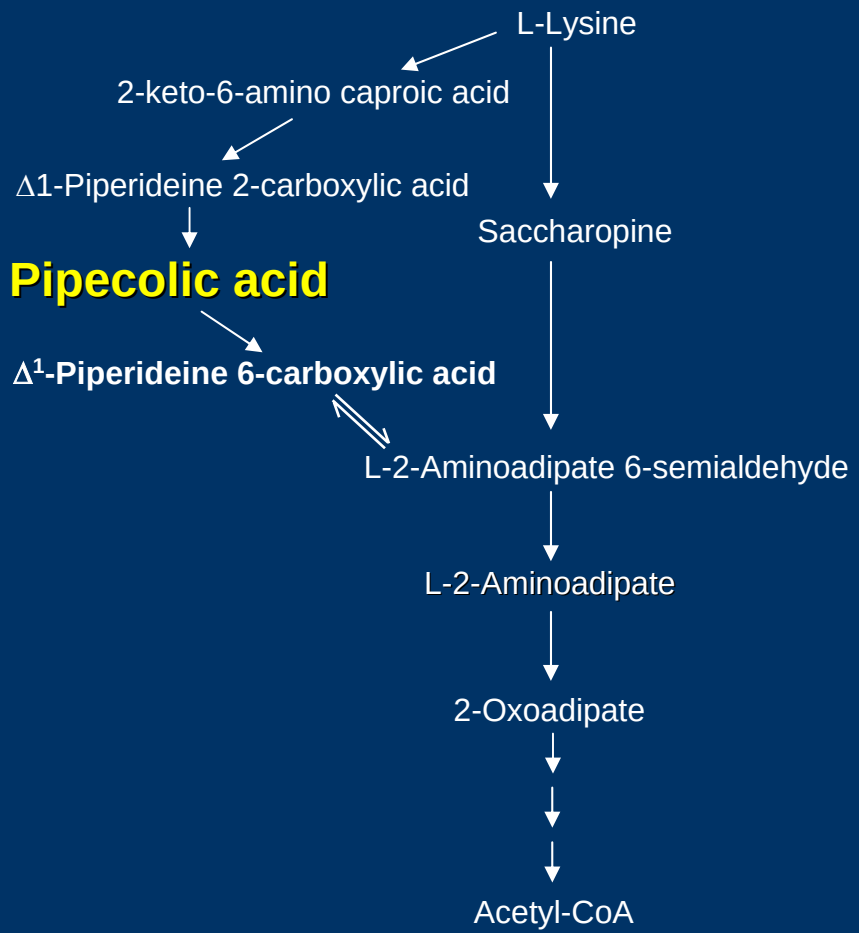
Pyridoxine-dependent seizures



Δ^1-Piperideine-6-carboxylic acid
P6C



L-Δ^1-Pyrroline-5-carboxylic acid
P5C



Pyridoxine-dependent seizures

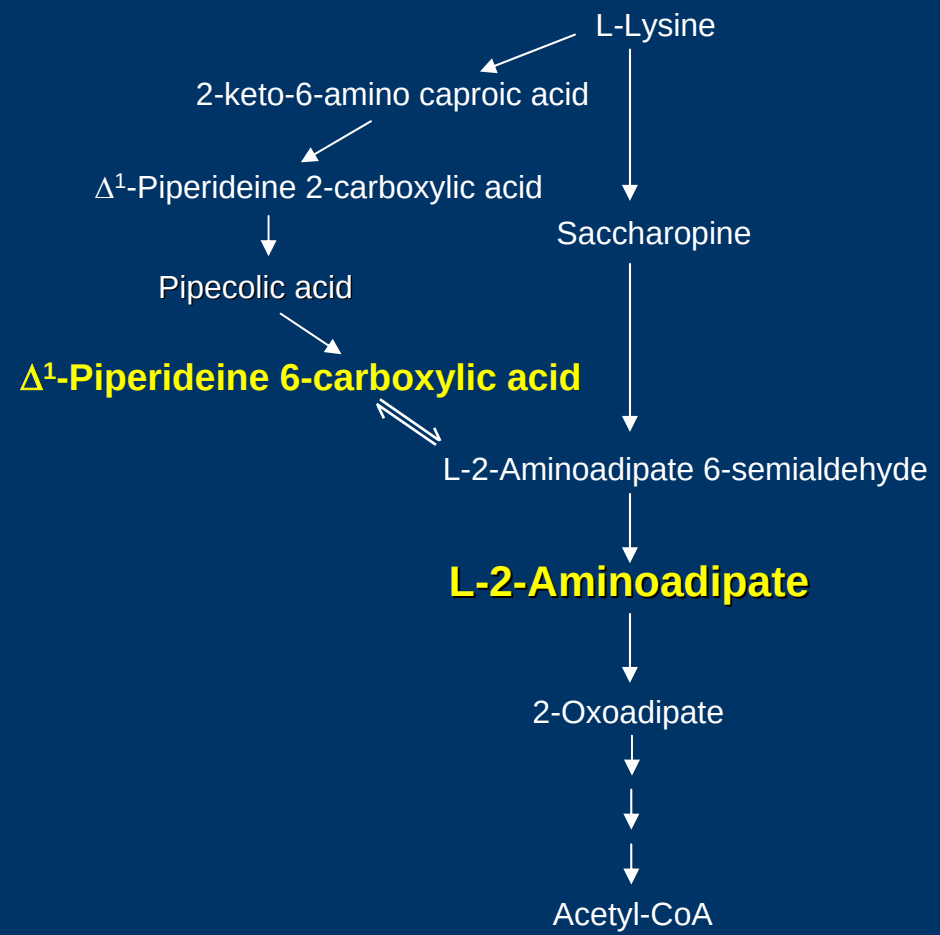
P6C dehydrogenase (*S. clavuligerus)*

FAAVGTAGQRCTTLRRL

C. elegans P6C; *P. sativum* P6C
B. subtilis P5C

ANTIQUITIN
(*ATQ1; ALDH7A1*)

Maps to 5q31



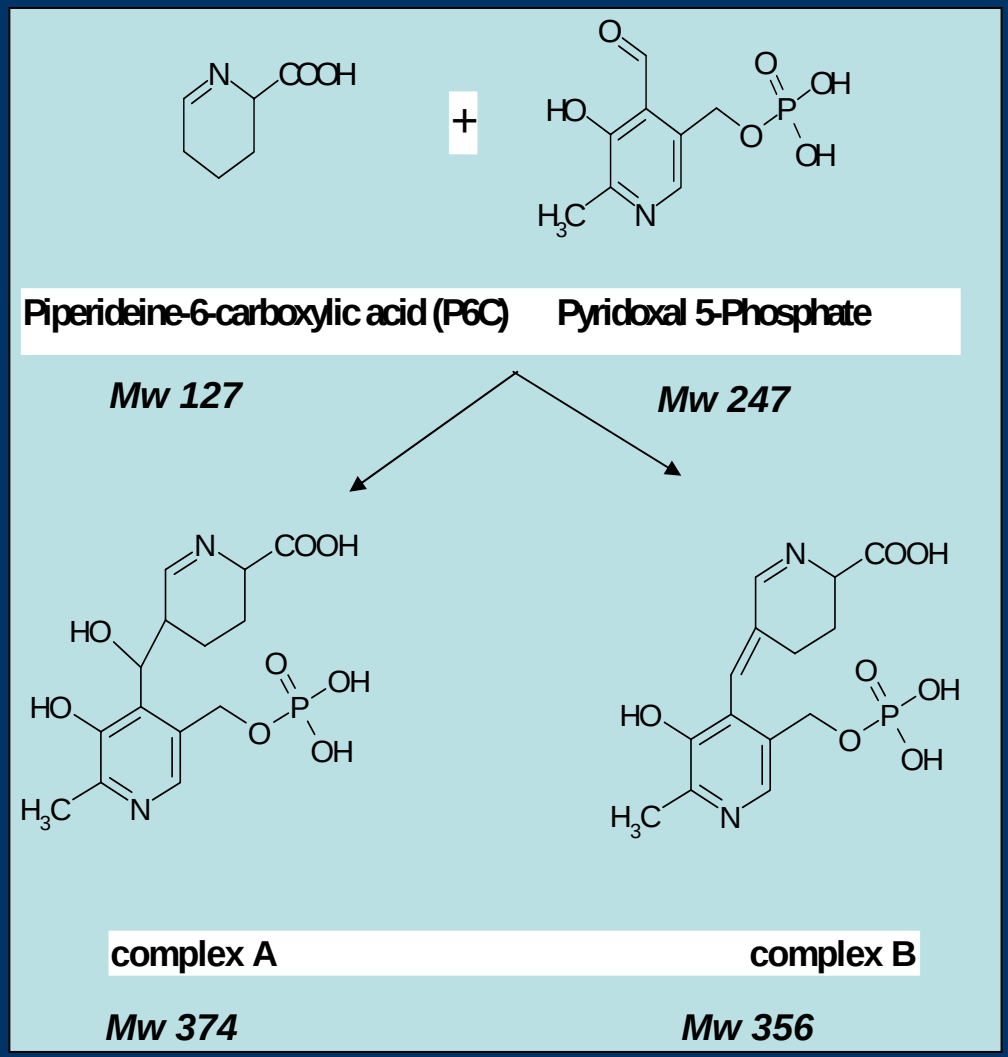
Antiquitin (ALDH7A1)

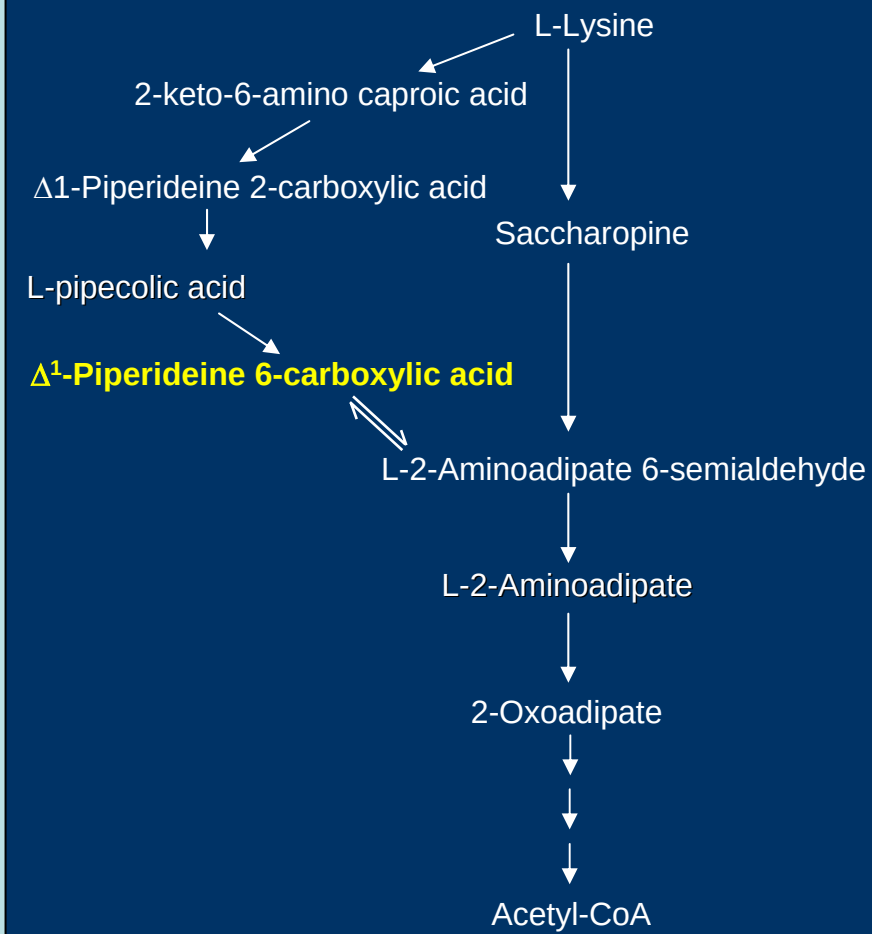
- First described in peas – cellular turgor pressure
- Highly conserved across species
- Belongs to the superfamily of aldehyde dehydrogenases
- Shown to have acetaldehyde dehydrogenase activity
- Exact physiological role has not previously been elucidated

Antiquitin (ALDH7A1)

- Mutations in the antiquitin gene cause pyridoxine-dependent epilepsy in man.

Pathophysiologic mechanism in PDE: analogy to hyperprolinemia type II



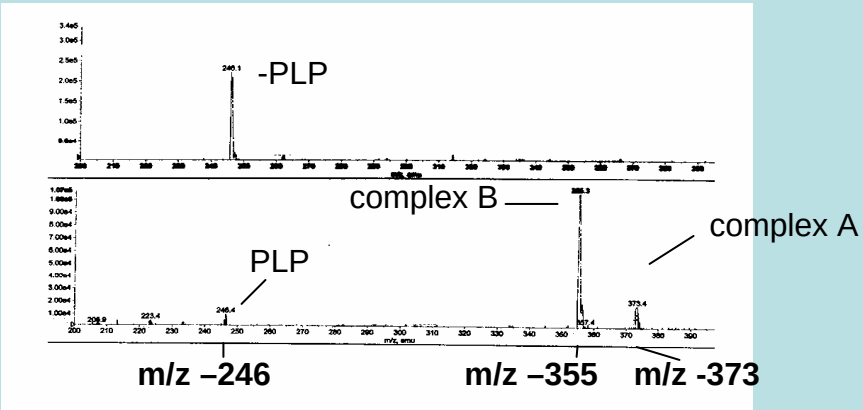


Pathophysiologic mechanism in PDE

P6C + PLP ⟶ **complex A + complex B**

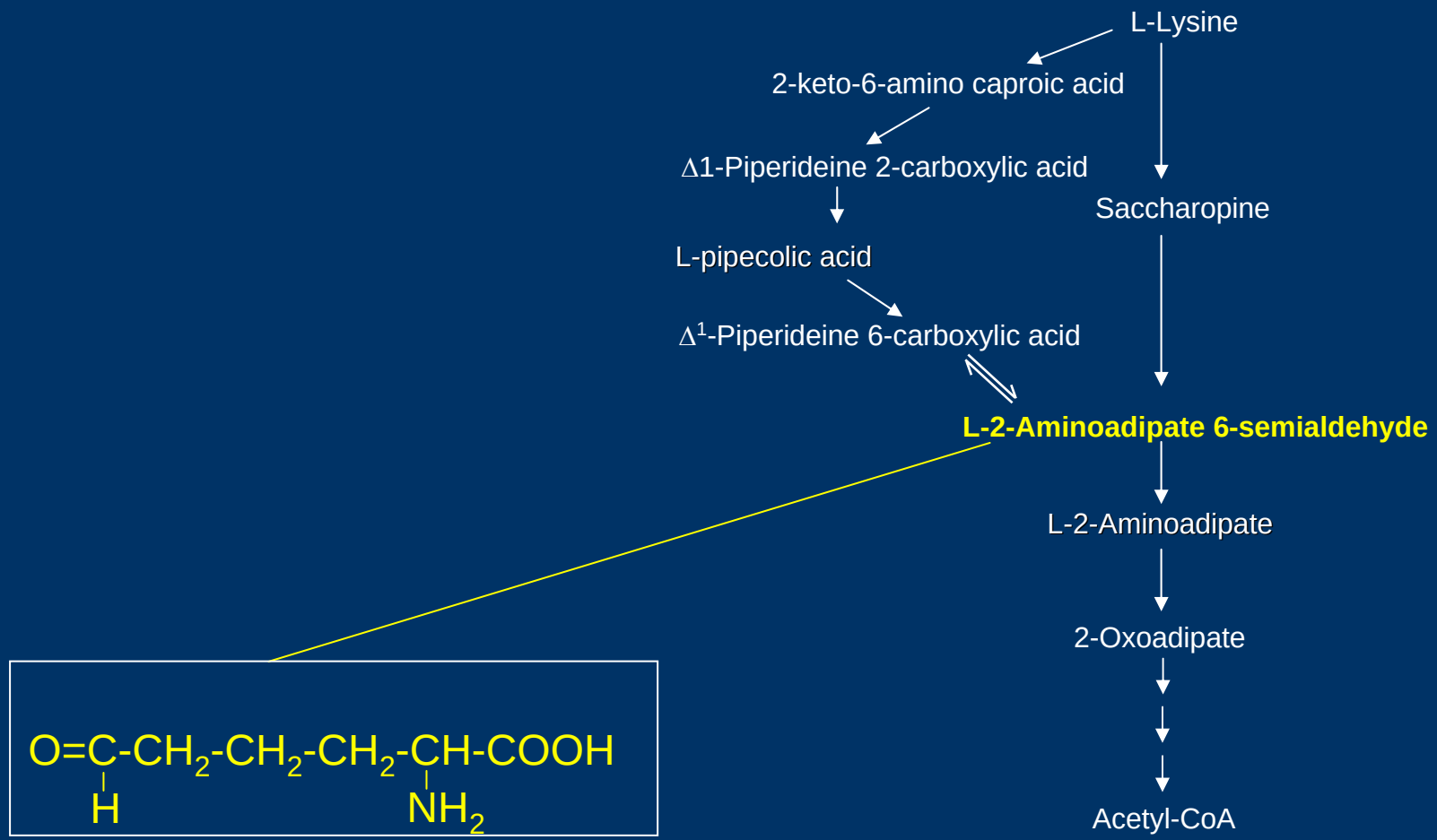
Experiment: Aqueous solution

1 mmol/L P6C
1 mmol/L PLP
pH 7.5
37°C, 24 hours



Hypothesis of PLP deactivation by complexation with P6C appeared to be true (*in-vitro*).

L-2-Aminoadipate 6-semialdehyde



L-2-Aminoadipate 6-semialdehyde determination

- Matrices: cerebrospinal fluid, plasma, and urine.

- ^{15}N-aminoadipic acid used as internal standard.

- Convert AASA and IS to corresponding FMOC-derivatives.

- Measurement performed by LC-MS/MS in MRM mode.

L-2-Aminoadipate 6-semialdehyde determination

AASA in bodyfluids of patients with pyridoxine-dependent seizures
Cohort 1

	patients	controls
CSF *(µmol/L)* 3/12 available	1; 28; 19	<0.1
plasma *(µmol/L)* 5/12 available	3.5 ± 1.2 *range 1.5 – 4.6*	<0.2
urine *(mmol/mol creat.)* 9/12 available on treatment 1/12 available before treatment	14 ± 8 *range 8 – 28* 168	<1

L-2-Aminoadipate 6-semialdehyde a novel biomarker for PDE?

Increase of biomarker in bodyfluids of PDE patients
2 cohorts

	AASA	L-pipecolic acid
CSF	↑↑	↑↑
plasma	↑↑	↑
urine	↑↑	nl.-↑ *

* nl. on-therapy, elevated off- (before) therapy

Yes, a non-invasive specific diagnostic metabolite for PDE, independent whether patients are on/off therapy!

CONCLUSIONS

AASA is consistently increased in body fluids derived from PDE patients (now found in two cohorts).

CONCLUSIONS

AASA is consistently increased in body fluids derived from PDE patients.

Since AASA is in equilibrium with P6C, P6C is expected to be increased.

CONCLUSIONS

AASA is consistently increased in body fluids derived from PDE patients.

Since AASA is in equilibrium with P6C, P6C is expected to be increased.

P6C complexate with PLP in-vitro: supporting the hypothesis of PLP-deactivation in PDE.

CONCLUSIONS

AASA is consistently increased in body fluids derived from PDE patients.

Since AASA is in equilibrium with P6C, P6C is expected to be increased.

P6C complexate with PLP in-vitro: supporting the hypothesis of PLP-deactivation in PDE.

Increases of AASA revealed that antiquitin acts on AASA, and that the metabolic impairment is located at the level of the conversion of AASA into alpha-aminoadipic acid.

CONCLUSIONS

AASA is consistently increased in body fluids derived from PDE patients.

Since AASA is in equilibrium with P6C, P6C is expected to be increased.

P6C complexate with PLP in-vitro: supporting the hypothesis of PLP-deactivation in PDE.

Increases of AASA revealed that antiquitin acts on AASA, and that the metabolic impairment is located at the level of the conversion of AASA into alpha-aminoadipic acid.

AASA is a novel non-invasive diagnostic metabolite for PDE.

No need for test of pyridoxine withdrawal.

Participants

Cornelis Jakobs

Philippa Mills
Peter Clayton

Michel AAP Willemsen
Levinus A Bok

Barbara Plecko
Peter Baxter
Matthias Baumgartner
Heymut Omran
Uta Tacke
Birgit Uhlenberg
Berhard Weschke

Kerra Pearce
Liz Bland