Brain mitochondria isolation and respiration protocols

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Brain tissue specificity

- Different types of the cells (neurons and glial)
- Different zones have different composition of neurons and glial cells
- Brain tissue is rich in membranes
- Synaptosomes are artificial, membranous sacs that contain synaptic components and are generated by subcellular fractionation of homogenized or ground-up nerve tissue
- Synaptic and non-synaptic mitochondria subpopulations

Brain mitochondria isolation protocol

Total brain mitochondria fraction with little contamination from synaptosomes and myelin

Medium, pH 7,4:
250mM Sucrose
10mM Tris/HCl
0,5 mM EGTA

Homogenization: glass-teflon

Centrifugation
1000g – 5 min.
7000g – 10 min.

3% and 6% Ficoll gradient
11500g – 30 min.

Ficoll can be changed by percoll or suchrose.

Conventional brain mitochondria isolation protocol

Brain mitochondria isolation protocol

Total brain mitochondria fraction

Medium, pH 7,4 :
225mM Mannitol
75mM Sucrose
5mM Hepes
1mM EGTA

Homogenization: glass-teflon

Centrifugation
1000g – 5 min.
7000g – 10 min.

Brain mitochondria isolation protocol

Fraction containing synaptic and non-synaptic mitochondria

Medium , pH 7,4:
225 mM Mannitol
75 mM Sucrose
1 mM EGTA
5 mM HEPES
1 mg/ml BSA
0.05% Nagarse

Homogenization: glass-glass

Centrifugation
2000 g - 4 min.
12 000 g - 9 min.

To permeabilize synaptosomes pelet
homogenised in medium with 0.02% digitonin
12000 g - 11 min.

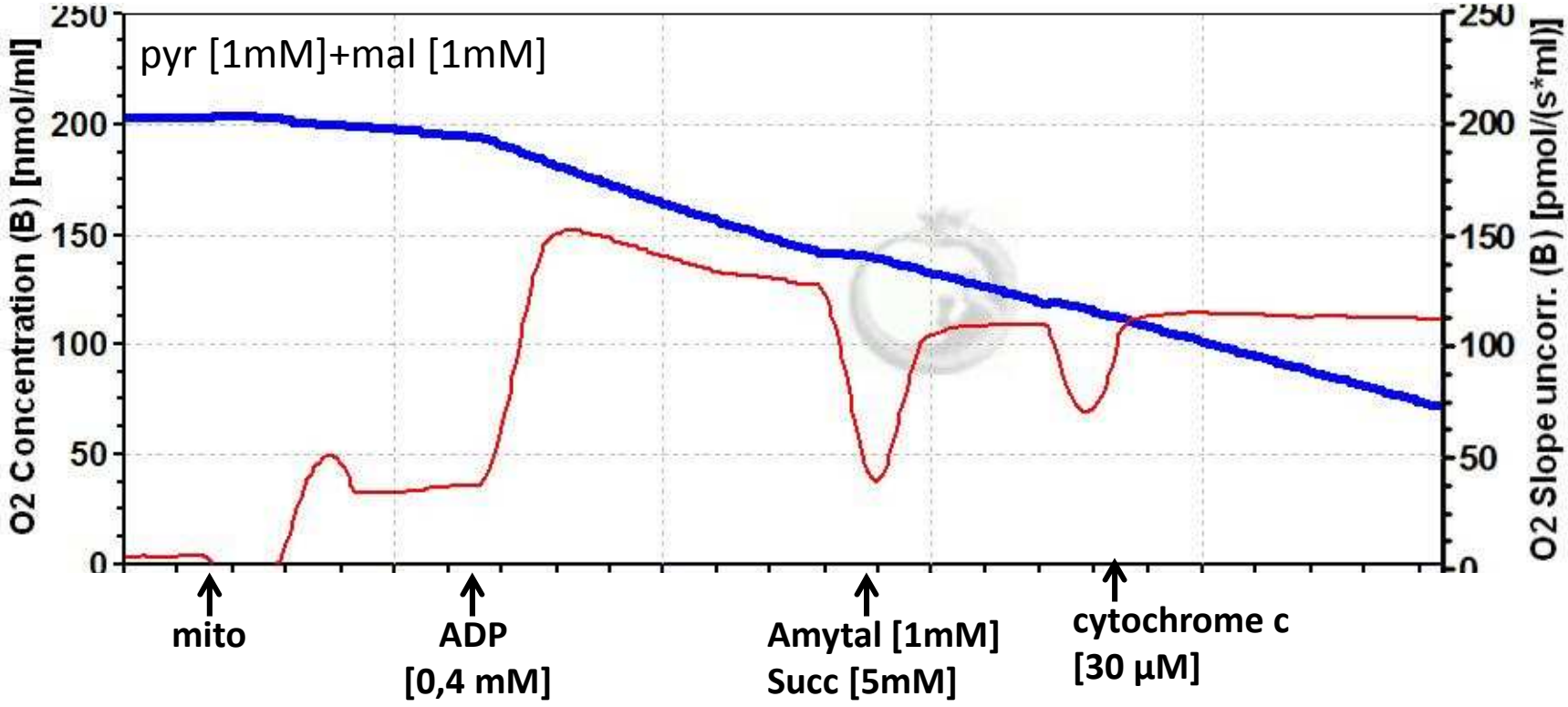
Kudin AP, 2004 J Biol Chem. Feb 6;279(6):4127-35.

Brain mitochondria respiration protocol

Medium:

110mM KCl, 2,24mM $MgCl_2$, 5mM KH_2PO_4, 10mM Tris HCl

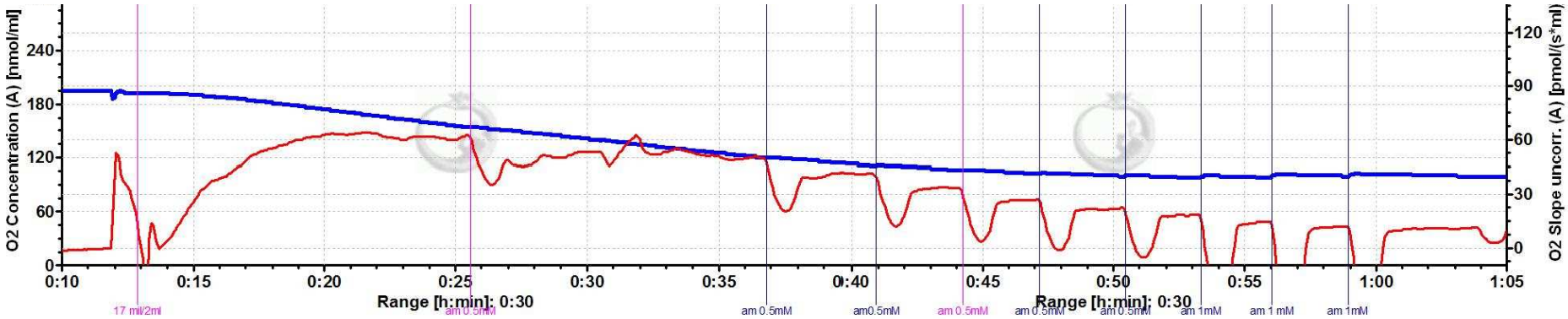
Total cerebral cortex mitochondria fraction 0,25mg/ml



Oxygen consumption protocol

Medium: DMEM (glucose)

Neurons isolated from cerebellum 8,5 mln./ml



Titration with amytal [0,5mM]