CHARACTERIZATION OF THE PROTECTIVE EFFECTS OF ADENINE NUCLEOTIDES ON PERMEABILITY TRANSITION IN ISOLATED BRAIN MITOCHONDRIA

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INTRODUCTION

Excessive mitochondrial Ca^{2+} uptake can cause non-selective inner mitochondrial membrane permeabilization, known as permeability transition (PT). This event can lead to apoptosis, since these organelles contain proteins involved in this process such as cytochrome *c*, the apoptosis inducing factor and pro-caspases. PT is involved in neurodegeneration followed by hypoglycemia, brain ischemia and trauma. The adenine nucleotides, ADP and ATP, are probably the most important endogenous inhibitors of mitochondrial PT (Vercesi et al., Front Biosci, 2006, 11, 2554-64). The aim of the present study was to characterize the inhibitory effect of ADP and ATP on Ca^{2+}-induced PT in isolated rat brain mitochondria.

METHODS

→**Isolation of rat brain mitochondria**: Non-synaptosomal mitochondria were isolated by the Percoll gradient separation method (Sims, J Neurochem, 1990, 55, 698-707), with some modifications (Kristián et al., J Neurochem, 2002, 83, 1297-308).

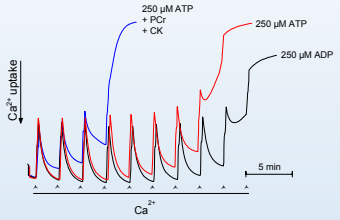
→**Measurements of Ca^{2+} uptake:** Ca^{2+} uptake by isolated mitochondria was determined using the Calcium Green 5N, a fluorescent probe, with excitation and emission wavelengths of 506 and 532, respectively (Murphy et al., Proc Natl Acad Sci USA, 1996, 93, 9893-8).

→**Measurements of oxygen uptake**: Oxygen consumption was measured using a computer-interfaced Clark-type electrode from Hansatech Instruments (Hansatech Instruments, England) in standard reaction medium (130 mM KCl, 10 mM K^+ - HEPES buffer (pH 7.2), 200 µM EGTA, 2 mM P_i, 1 mM Mg^{2+}, 5 mM malate and 5 mM glutamate) at 37°C in a sealed glass cuvette equipped with magnetic stirrer.

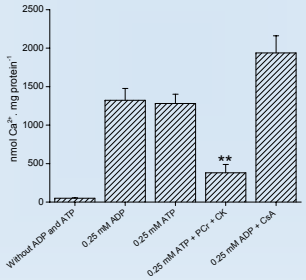
→**Determination of mitochondrial swelling**: Mitochondrial swelling was estimated through light scattering changes at excitation and emission wavelengths of 540 nm (Maciel et al., J Neurochem, 2004, 90, 1025-35).

RESULTS

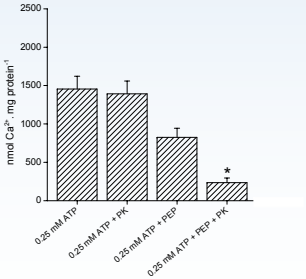
Mitochondrial Ca^{2+} accumulation was similar in the presence of either ADP or ATP. Interestingly, ATP lost most of its inhibitory properties when the experiments were carried out in the presence of the ATP-regenerating systems phosphocreatine (10 mM PCr)/creatine kinase (20 µg/mL CK).



In the absence of ADP and ATP, isolated rat brain mitochondria showed low Ca^{2+} accumulation capacity. In the presence of either ADP or ATP at 0.25 mM, mitochondria accumulated approximately 1500 nmol Ca^{2+} X mg protein^{-1}. Interestingly, in the presence of ATP, PCr and CK, the amount of Ca^{2+} accumulation was about 70% lower.

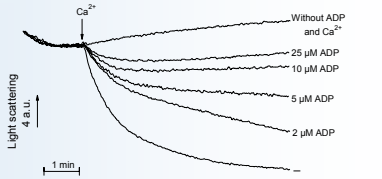


The experiments with 0.25 mM ATP and 9 µg/mL pyruvate kinase (PK) showed that this enzyme has no significant effect on the PT induction. However, 2 mM phosphoenolpyruvate (PEP) increased the Ca^{2+} effect on the PT induction (Roos et al., FEBS Lett, 1978, 94, 418-21). Interestingly, in the presence of 0.25 mM ATP, PEP and PK, the amount of Ca^{2+} accumulation was about 71% lower than with 0.25 mM ATP and PEP.

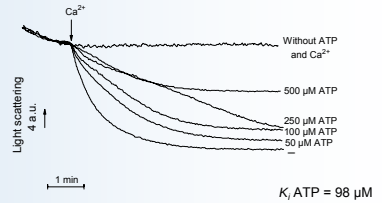


These results indicate that PT inhibition observed in the presence of added ATP could be mainly due to hydrolysis of ATP to ADP.

K_i values for ADP and ATP determined by the rate of Ca^{2+}-induced mitochondrial swelling were 4.5 and 98 µM, respectively.



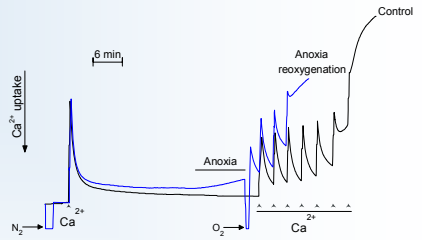
K_i ADP = 4.5 µM



K_i ATP = 98 µM

After a period of anoxia/reoxigenation, mitochondria incubated in the presence of 0,25 mM ADP were more susceptible to PT.

This result indicates that anoxia/reoxigenation decreases the inhibitory effect of ADP on PT.



CONCLUSION

We conclude that ADP is a potent inhibitor of brain mitochondrial PT, while ATP is a weaker inhibitor of this phenomenon.

The inhibitory effect of ADP on PT is partially lost after anoxia/reoxigenation.

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