Kinetic Model of AMPK-mediated Autophagy as Excitotoxic Stress Response

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We developed a deterministic, kinetic model of AMPK-mediated (macro)autophagy as response to excitotoxic stress in mammals. We used ordinary differential equations to describe the dynamic behavior of the system and mass action and Michaelis-Menten kinetics to specify the reaction rates. COPASI 4.27[1] was used to define the system and to run time-course simulations. The kinetic parameters of our model are based on values from two existing, kinetic models[2,3]. The first model describes AMPK-regulated autophagy initiation[3]. The second model simulates AMPK-mediated neuroprotection against excitotoxicity[2]. Our model combines two crucial pathways that determine neuronal cell fate.

Introduction

Non-selective macroautophagy

- response to alterations of extra- and intracellular conditions
- degrades specific molecules, components and sets free missing metabolites
- contributes to longevity of cells[7]
- two sensor proteins: AMPK (AMP-activated protein kinase) and mTORC1 (mammalian target of rapamycin)
- AMPK maintains energetic and metabolic homeostasis[2,3] and senses energy levels via the AMP:ATP ratio[2]

Excitotoxicity

- caused by ischemia stroke, head trauma or septic shock[4,5]
- pathological, excessive stimulation of glutamate receptors[2]
- high levels of cytosolic Ca^{2+} cause energy deprivation
  → neuroprotection via AMPK-mediated, glucose-dependent ATP production[2]
- severe excitotoxicity induces necrosis[2]
- cells can trigger delayed apoptosis[5], after reestablishment of ion homeostasis
- excitotoxicity can induce autophagy[4,6]

- autophagy and AMPK may have negative effects on neuronal survival[5,6]
- we developed a kinetic model of excitotoxicity-induced, AMPK-mediated autophagy in neurons
  → our model predicts induction of autophagy after reestablishment of ATP levels
- neuronal injury can result in irreparable, neurological disabilities, an effective treatment is still missing[4]
- important to further investigate if autophagy is a side effect of an apoptotic cell, or the trigger of programmed cell death

Results

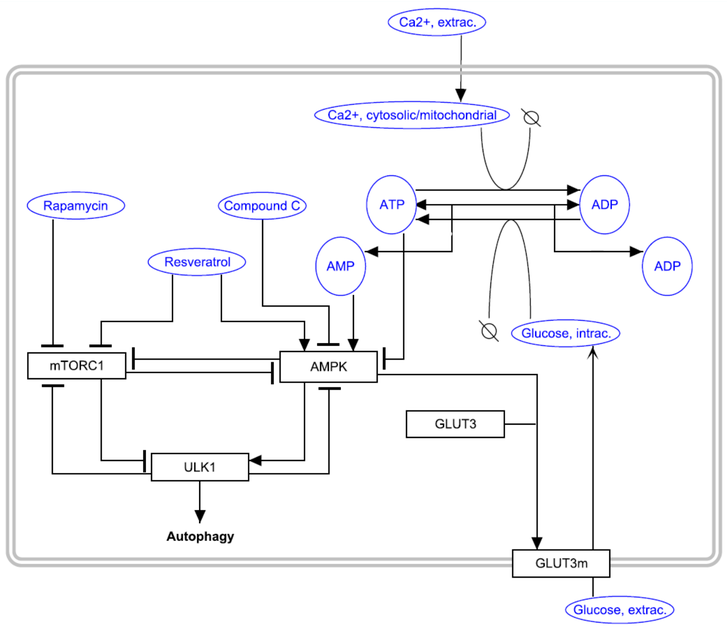

Fig. 1: Neuronal response to excitotoxic stress and regulation of autophagy initiation: Excitotoxic stress is mimicked by high Ca^{2+} levels. ATP level deprivation activates AMPK. AMPK mediates energy level restoration and neuroprotection[2]. Rapamycin, resveratrol and high AMP levels induce autophagy. Compound C inhibits AMPK and autophagy. AMPK activates ULK1 (Unc-51-like kinase1), promotes autophagy and inhibits mTORC1. The treatment with different combinations of drugs mimics different metabolic conditions[3].

Moderate Excitotoxicity Induces no Autophagy

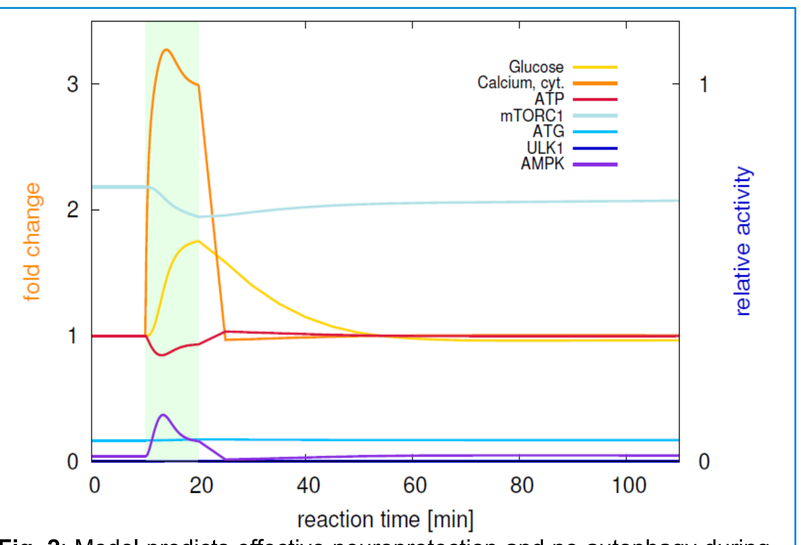

Fig. 2: Model predicts effective neuroprotection and no autophagy during moderate excitotoxicity: Excitotoxicity is mimicked by an increased Ca^{2+} influx for 10 min. AMPK-mediated neuroprotection restores ATP levels. Moderate activity of AMPK is not sufficient to activate ULK1 or induce autophagy (ATG, autophagy-related genes). All metabolic (orange) concentrations are normalized to baseline (left y-axis). Concentrations of active proteins (blue) are given in relative activity (right y-axis).

Prolonged Excitotoxicity Induces Autophagy

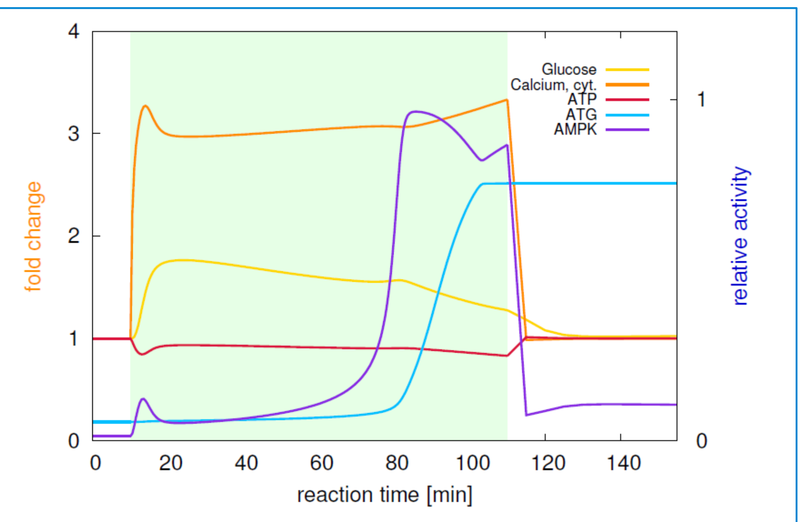

Fig. 3: Model predicts strong activation of AMPK and induction of autophagy during prolonged excitotoxicity: Excitotoxic stress is mimicked by an increased Ca^{2+} influx for 100 min. AMPK-mediated neuroprotection restores ATP levels. Active AMPK inhibits mTORC1 and activates ULK1 and autophagy (ATG).

Severe Excitotoxicity Induces Delayed Autophagy

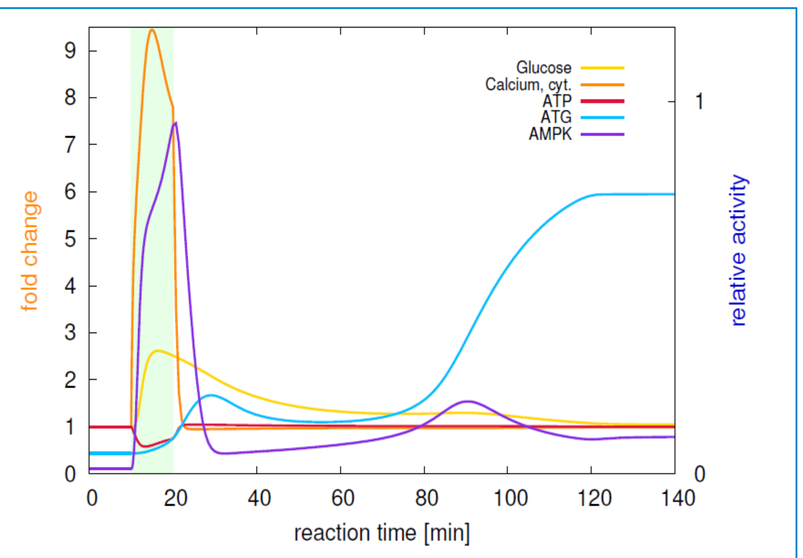

Fig. 4: Model predicts activation of AMPK and delayed autophagy after severe excitotoxicity: Excitotoxic stress is mimicked by a highly increased Ca^{2+} influx for 10 min. AMPK-mediated neuroprotection restores ATP levels. Although all concentrations return to the steady state, autophagy is induced.

Conclusion

Our model simulates the pathways of excitotoxic stress response and autophagy initiation and predicts a direct cause-and-effect behavior. The model proposes that:

- moderate excitotoxicity induces no autophagy (moderate and temporary activation of AMPK is probably not sufficient to induce autophagy)
- prolonged excitotoxicity induces autophagy
- severe excitotoxicity induces delayed autophagy, despite successful neuroprotection and return to the steady state

Further experiments could investigate the qualitative and quantitative predictions of the model. Deeper understanding of the negative role[5,6] of autophagy and AMPK in excitotoxic stress response may help to predict:

- if a neuron survives excitotoxic stress or induces delayed apoptosis
- **if autophagy is observed as delayed effect of excitotoxic stress**
- **if delayed autophagy is mainly a side effect or actually the cause for apoptosis**

Deciphering the negative role of autophagy and AMPK could yield new targets for excitotoxicity treatment. Due to a limited time frame of two months (Bachelor's thesis), we propose the following methodical improvements for further work on our model. Steady state analysis, elimination of the Michaelis-Menten approximation (if possible), construction of a corresponding Petri net and knock-out analysis are possible next steps.

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