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Title:
Spatiotemporal metabolic organization during development of brain cell cultures

Abstract

The brain requires a large amount of energy for proper functioning which constrains an increase of neuronal communication due to limited energy supply. This problem may be circumvented by optimizing the neuronal connection instead of increasing the number of neurons. Such a fine reorganization of the neuronal network is established during early postnatal brain development.

In this thesis, the developmental changes of the energy metabolism during neuronal network development have been investigated in order to get deeper insight into the impact of the energy metabolism for optimization of neuronal communication. Hippocampal cell cultures, containing both neurons and glia cells, were incubated in nutrition containing medium for the first three weeks *in vitro*. In this interval, the energy metabolism during neuronal network formation in rat hippocampal cell cultures has been studied by means of fluorescence imaging together with chemical stimulation. NAD(P)H and intracellular protons have been taken as a measure for the energy metabolism because they are produced by glycolysis and subsequently consumed by mitrocondrial respiration. NAD(P)H was recorded by its autofluorescent and protons were detected by using a suitable fluorescent pH-indicator dye.

Developmental changes of the energy metabolism were investigated. This process was traced by recording NAD(P)H fluorescence in response to NMDA-induced activation of neuronal activity. We observed progressive changes of stimulation evoked NAD(P)H signaling during the first three weeks *in vitro*. At day 7 and 16, this response was minimal, yielding a biphasic pattern that reproduced earlier findings of about developmental changes of population spike (a shift in electrical potential as a consequence of the movement of ions involved in the generation and propagation of action potentials) amplitudes or glutamate release in young rats. Inhibition of mitochondrial respiration by KCN revealed that the NMDA-evoked stimulation of the energy metabolism is mainly due to an increase in glycolytic activity.

Imaging of intracellular pH (pH_i) revealed traveling proton waves which induced by blocking mitochondrial respiration with cyanide. These waves were accompanied by network oscillations in pH_i. During the observation period (3-22 day *in vitro* (DIV)), most of the observed cells showed a biphasic response which are characterized by an initial acid shift and subsequent alkalinization. It was during this alkalinization period that concomitant waves and network oscillations could be observed, however, only after 14 DIV. NMDA induced similar changes in pH_i except that propagating waves could not be observed. Our results indicate that the energy metabolism of hippocampal cells undergoes age-dependent dynamic instabilities leading to the formation of traveling proton waves.