ASTROCYTIC COMMUNICATION AND CELL DEATH DURING METABOLIC DEPRESSION AND OXIDATIVE STRESS

Christina Nodin
Institute of Neuroscience and Physiology at Sahlgrenska Academy, University of Gothenburg, Sweden, 2008

Abstract
Stroke is a major cause of death and adult disability in the western world. Most often, stroke is caused by the occlusion of a brain artery. Within the perfusion territory of the occluded vessel, various degrees of necrotic and delayed programmed cell death will occur if the occlusion persists, leading to expanding tissue damage. Astrocytes are the most numerous cells in the brain, but the astrocytic response to ischemic conditions and the extent to which these cells can recover after an ischemic insult is not well understood. An increasing amount of evidence indicates that astrocytes are more sensitive to ischemic injury than previously thought. Astrocytic functions are vitally important for neuronal activity during physiological conditions and probably during various pathological situations, including stroke. Astrocytes are highly coupled by intercellular gap junction channels that enable the formation of large cellular networks. These networks provide the basis for several important astrocytic functions including intracellular signalling and transport of molecules and metabolites.

In order to investigate astrocytic reactions during metabolic depression we used the glycolytic blocker iodoacetate (IA) in primary astrocyte cultures. This treatment induced a reproducible and concentration-dependent ATP decrease which was associated with a profound increase in the activity of reactive oxygen species (ROS). This suggests that metabolic depression induced oxidative stress. Moreover, programmed cell death was initiated in individual astrocytes or small cell clusters and spread to include large clusters of astrocytes. However, when gap junction communication was inhibited during metabolic depression, programmed cell death was initiated in individual cells but no expansion into large cell clusters was observed. This suggests that gap junction permeable substances contribute to the spreading of cell death in astrocytes. The observed programmed cell death involved translocation of apoptosis inducing factor from the mitochondria to the nucleus. Similar results were observed in a model of oxidative stress using 3-morpholinosyndomine (SIN-1), a compound known to produce equimolar amounts of superoxide and nitric oxide which react to form peroxynitrite. Caspase-activation was not observed in astrocytes exposed to metabolic depression or oxidative stress.

Astrocytes and several other cell types express endogenous antioxidant systems. The expression of many of the enzymes involved in this cellular defense is regulated by the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2). The potential protective effect of the Nrf2 system in astrocytes was investigated by using the Nrf2-activating phytochemicals sulforaphane (naturally occurring in broccoli) or curcumin (from turmeric), or the commonly used food additive tert-butylhydroquinone. Exposing the astrocytes to these substances before adding IA or SIN-1, prevented the oxidative stress, enabled the astrocytes to maintain their ATP levels and efficiently prevented cell death. Similar results were observed when the exogenous ROS scavengers trolox (a vitamin E analogue), tempol (a superoxide dismutase analogue) or the free radical scavenger cocktail B27 were used.

Finally, we investigated the possibility for the astrocytes to recover following a simulated reperfusion injury where metabolic depression was reversed by washing out IA. Although metabolic depression was interrupted early during the ATP decrease, the astrocytes were not able to recover their ATP levels and widespread cell death occurred. However, pre-treatment with Nrf2 activators or addition of exogenous ROS scavengers enabled recovery of ATP levels and prevented cell death.

In summary, these results show that astrocytic cell death mediated by metabolic depression and oxidative stress involves the translocation of apoptosis inducing factor. In addition, gap junction communication was important for the spreading of cell death during metabolic depression. Finally, astrocytes were efficiently protected by activation of Nrf2-regulated endogenous antioxidant systems, which may represent an interesting target for the limitation of ischemic injury.

Key words: astrocyte, iodoacetate, SIN-1, gap junction, ATP, ROS, oxidative stress, Annexin V, AIF, programmed cell death, Nrf2, scavenger

ISBN 978-91-628-7437-7

ASTROCYTIC COMMUNICATION AND CELL DEATH DURING METABOLIC DEPRESSION AND OXIDATIVE STRESS

Akademisk avhandling

som för avläggande av medicine doktorsexamen
vid Sahlgrenska akademin vid Göteborgs universitet
kommer att offentligen försvaras i hörsal Arvid Carlsson,
Medicinaregatan 3, fredagen den 4 april 2008 kl 09.00

av
Christina Nodin

Fakultetsopponent:
Professor Carsten Culmsee, Philipps-Universitaet Marburg, Tyskland

Avhandlingen baseras på följande delarbeten:

I. Nodin, C., Nilsson, M. and Blomstrand, F.
Gap junction blockage limits intercellular spreading of astrocytic apoptosis induced by metabolic depression.
Journal of Neurochemistry 2005, Aug;94(4):1111-23.

II. Nodin, C., Nilsson, M. and Blomstrand, F.
Exogenous free radical scavengers or activation of Nrf2-regulated defense systems prevent astrocytic cell death induced by metabolic depression.
Manuscript

III. Nodin, C., Zhu, C., Blomgren, K., Nilsson, M. and Blomstrand F.
Metabolic depression and oxidative stress induce astrocytic cell death involving translocation of apoptosis inducing factor.
Manuscript

GÖTEBORGS UNIVERSITET