Insight into the Protective Mechanisms of Cortical Spreading Depression in Cardiac Arrest Cerebral Ischemia in Rat

N. Kawahara¹, C.A. Ruettzler¹, G. Mies², S.D. Croll³, S.J. Wiegand³, and I. Klatzo¹

Summary

Cortical spreading depression has recently been shown to protect hippocampal CA1 pyramidal neurons, as well as cortical neurons, against ischemic damage if elicited prior to ischemia. The present study was undertaken to investigate pathomechanisms of neuronal protection conferred by cortical spreading depression (CSD) elicited by the topical application of 5M KCl to the occipital cortex in Sprague-Dawley rats. Autoradiographic analysis on brain tissue metabolism revealed an increase of cerebral protein synthesis 3 days after CSD, whereas a reduction of glucose utilization was observed in both the CSD and control (NaCl application) groups. Immunohistochemistry disclosed an enhanced expression of c-Fos, glial fibrillary acidic protein (GFAP), and basic fibroblast growth factor after CSD, whereas heat-shock protein (hsp) 72 was not induced throughout an observation period of up to 7 days. Northern blot analysis for brain-derived neurotrophic factor (BDNF) revealed a biphasic increase in mRNA, the first peak appearing at 4h and the second at 3 days after CSD. Quantitative measurement of BDNF by enzyme linked immunosorbent assay (ELISA) was in accordance with the biphasic pattern of mRNA, although the second peak lasted up to 7 days. These results show that neither metabolic suppression nor induction of hsp72 are related to postischemic neuronal protection following CSD, and suggest that stimulation of neurotrophic factors prior to an ischemic insult is a possible mechanism for neuronal resistance.

Introduction

Measures to ameliorate ischemic brain damage have been pursued for many years. Following the discovery that preconditioning by short sublethal cerebral ischemia can be protective against a subsequent lethal period of ischemia - a phenomenon known as “ischemic tolerance” [4, 5] - extensive studies have been centered on the elucidation of endogenous mechanisms for protection against ischemia. In a previous study, we demonstrated that cortical spreading depression (CSD) elicited 3 days prior to

¹ Stroke Branch, National Institutes of Health, Bldg 36, Rm4A-03, 36 Convent Drive, MSC 4128, Bethesda, MD 20892-4128, USA
² Max-Planck Institute for Neurological Research, Glueler Str. 50, 50931 Cologne, Germany
³ Regeneron Pharmaceuticals Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591, USA
lethal ischemia could also induce a tolerant state in hippocampal CA1 pyramidal neurons [3]. This effect was not observed when CSD was induced 1 or 7 days prior to ischemia. Protection of cortical neurons was further confirmed 24 h after CSD [6]. The purpose of the present study was to elucidate changes in brain tissue associated with transient neuronal protection following transient CSD, with special reference to alterations in brain metabolism and changes in expression of hsp72 and growth factors, such as basic fibroblast growth factor (bFGF) and brain-derived neurotrophic factor (BDNF).

Materials and Methods

Cortical spreading depression was elicited in Sprague-Dawley rats by topical application of 5 M KCl to the exposed dura on the left occipital cortex for 1 h as described previously [3]. Sham operated rats and rats in which KCl was substituted by NaCl of equal concentration served as controls.

Altered alterations in metabolism were assayed using autoradiographic analyses for glucose utilization and protein synthesis in rats sacrificed 3 days following CSD by use of a double tracer technique (14C deoxyglucose and 3H-leucine, n = 4).

Immunohistochemical evaluation of c-Fos, hsp72, glial fibrillary acidic protein (GFAP), and bFGF was carried out in rats sacrificed at 3 h, and 1, 3 and 7 days following induction of CSD (n = 4 in each group), and brains were processed for immunohistochemical analysis using an antibody against c-Fos (Oncogene Science), hsp72 (Amersham), GFAP (Sigma), and bFGF (Oncogene Science).

In another group of rats the expression of BDNF mRNA was examined by Northern blot analysis and in situ hybridization. Cortical and hippocampal total RNA from each hemisphere was isolated at 4 h and 24 h, and 3 and 7 days following either KCl or NaCl application, and hybridized to a 32P-labeled cDNA probe for BDNF. In in situ hybridization studies, fixed brain sections at the same time intervals were hybridized to a 35S-labeled cDNA probe for BDNF and exposed to autoradiographic film for several days. For quantitative measurement of BDNF, its protein content was measured using two-site enzyme linked immunosorbent assay (ELISA) in cortical and hippocampal homogenates taken at 4 h, 12 h and 24 h, and 3 and 7 days after electrolyte application (n = 4 in each group).

Results

Evaluation of glucose utilization and cerebral protein synthesis on the same section disclosed a marked reduction of glucose utilization in the ipsilateral hemisphere 3 days after KCl application, while cerebral protein synthesis was found to be discretely increased. A reduction of glucose utilization in the ipsilateral hemisphere was also observed following NaCl application, but cerebral protein synthesis remained unchanged (Fig. 1).

Immunohistochemical analysis for c-Fos exhibited conspicuously enhanced immunostaining of neurons throughout the ipsilateral cortex and bilaterally in the dentate gyri of the hippocampus only in rats subjected to CSD and sacrificed 3 h after