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Gene Expression and the Response to Ischemic Insults in Brain: Studies in a Gerbil Model of Induced Tolerance

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Abstract

Brief ischemic insults protect against injury following subsequent longer and otherwise damaging periods of ischemia, although such protective effects have usually been quite variable. Selective changes in gene expression are considered good candidates for mediators of the tolerance phenomenon, and the stress protein, hsp72, has received considerable attention in this regard. In recent experiments we have monitored the interval of ischemic depolarization following brief periods of occlusion and have identified depolarization thresholds for tolerance, for injury and for various changes in gene expression. While hsp72 is clearly inducible after moderate insults that do not injure neurons, our results indicate that neuronal protection can be achieved by even milder insults that do not appreciably induce hsp72, but that do induce immediate-early genes of the Fos and Jun families with lower depolarization thresholds. In addition, these studies provide the basis for a reproducible model in which mechanisms of tolerance can be further studied.

Key words: ischemia, heat shock, immediate-early genes, ischemic tolerance

Introduction

Ischemic insults bring about dramatic changes in the expression of many genes in brain as well as in other tissues. Available data indicates that some of these responses are coordinately regulated, and several distinct patterns of expression can be recognized¹⁾. However, the challenge remains to establish how changes in the levels of specific mRNAs and proteins may be related to the complex pathophysiology of postischemic injury or protection. The phenomenon of induced ischemic tolerance provides an example of a complex response in which changes in gene expression are believed to play an important role^{2,3)}. This presentation will focus on recent studies comparing gene expression following global ischemia of varied duration to distinguish between responses to insults that produce injury vs. those that induce tolerance.

The proto-oncogene, c-fos, and the stress protein, hsp72, show overlapping distributions after global ischemia but they are strikingly dissociated after focal insults. Both are expressed throughout the major neuron populations of hippocampus and in other brain regions after global ischemia, with transient expression of c-fos and more prolonged hsp72 induction, especially in vulnerable neurons⁴⁾. In contrast, c-fos and many other "immediate-early" genes are induced throughout ipsilateral cortex after focal ischemia, while hsp72 is restricted to an area including the evolving infarct and tis

immediately adjacent territory^{5/7)}. The brief depolarization associated with spreading depression following focal injury is apparently sufficient to trigger induction of *c-fos* and many other genes, while signals closely associated with the potential for injury are required to induce *hsp72*. Preliminary studies in the rat identified distinct "depolarization thresholds" for various inducible genes after global ischemia⁸⁾. In the present experiments we have extended this work to the gerbil with an evaluation of additional mRNAs and have included a determination of thresholds for tolerance in this model.

Methods

Gerbils (female, 50–70 g) were anesthetized with 1–2% halothane in 30% O₂, 70% N₂ and fixed in a stereotaxic frame. A glass microelectrode filled with 2 M potassium acetate (10 μ m tip diameter, resistance 10–30 M Ω) was placed in each hippocampus and DC potential was monitored via an amplifier system (World Precision Instruments) and Macintosh computer using the Super Scope II data acquisition system. Ischemia was produced by bilateral carotid artery occlusion using a weighted suture loop, with varied occlusion times as indicated below for individual studies.

For ischemic tolerance studies the animals were subjected to an initial priming challenge that resulted in ischemic depolarizations ranging between 0 and 10 min duration, followed 48 h later by a test ischemic insult that produced 6.5–8.5 min depolarization. Animals were perfused fixed with 10% formalin, 10% acetic acid in methanol one week after the second insult, and neuron counts in the CA1 pyramidal layer were obtained from hematoxylin/eosin stained paraffin sections of dorsal hippocampus. Preliminary studies established that depolarizations longer than 6 min resulted in complete CA1 neuron loss in naive animals under the present experimental conditions.

In gene expression studies a single ischemic insult was produced, resulting in a range of depolarization intervals comparable to that obtained in the tolerance experiments. Animals were killed after 1 h recirculation and fresh frozen sections processed for in situ hybridization with ³⁵S-labeled oligonucleotide probes specific for *hsp72*, *c-fos*, *c-jun*, *junB* and *junD*. Autoradiograms were quantitated by video densitometry calibrated with simultaneously exposed ¹⁴C standards.

Results

Variations in the timing of depolarization and repolarization in individual hippocampi produced considerable variability in the duration of depolarization after a given ischemic interval. However, for a given hippocampus, both ischemic tolerance and changes in gene expression were well correlated with the measured depolarization interval. As shown in Fig. 1, some degree of ischemic tolerance was detectable after depolarizations as short as 1 min, but essentially complete protection was observed after 2.5–3.5 min. Depolarizations of 4 min or longer themselves produced CA1 injury and reduced tolerance (data not shown). The several immediate-early genes examined all showed induction thresholds comparable to that observed for tolerance, while increases in *hsp72* expression were most prominent in association with insults that resulted in ischemic injury (Fig. 1).

Discussion

These results demonstrate that a highly reproducible ischemic tolerance model can be obtained when the severity of each ischemic insult is monitored. Variations in ischemic depolarization times are evident between hippocampi in the same animal, and between ipsilateral cortex and hippocampus (data not shown), so that individual measurements in each region of interest are essential. The predictable relationship between tolerance and depolarization suggests that differences in the severity of priming insults account for most of the variability observed in previous tolerance studies^{2/3)}. While it is difficult to produce a specific depolarization interval in a given hippocampus, maximum tolerance is observed over a broad enough range that tolerant animals can be produced in sufficient numbers for most studies.

Fig. 1 Depolarization thresholds for ischemic tolerance and gene expression in CA1 neurons. Gerbils were subjected to varied intervals of carotid artery occlusion, followed by evaluation of ischemic tolerance or gene expression as described in the text. Plotted values represent mean values obtained for cell counts or hybridization signals in hippocampi that depolarized at the times indicated. Grouped data are pooled for the following depolarization intervals (min): 0 (no depolarization), 1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-4, 4-6, 6-10. Tolerance is given as the percent of CA1 neuron counts in control animals, while expression of a given gene is shown as percent of the maximum group value obtained.

With regard to the relationship between tolerance and ischemia-induced changes in gene expression, it is evident from these studies that striking tolerance can be observed under conditions that result in minimal hsp72 expression. Essentially similar results have been obtained in studies in which hybridizations were done at longer recirculation intervals, after which a somewhat lower threshold for hsp72 expression is observed (data not shown). These findings suggest that a prior heat shock response is not a critical component of induced tolerance mechanisms, although it is strongly induced after the second test insult, and the stress response appears to be potentiated in tolerant animals²⁽⁹⁾. More rapid protein synthesis recovery in tolerant CA1 neurons may contribute to more effective induction of hsp72 and other proteins encoded by ischemia-induced mRNAs¹⁰⁽¹¹⁾.

The several immediate-early genes induced in association with tolerance function as components of transcription factor complexes that regulate the expression of diverse target genes¹²⁾. Previous studies have demonstrated increases in Jun immunoreactivity in CA1 neurons during tolerance induction¹⁾, and recent results have specifically indicated preferential accumulation of c-Jun protein¹³⁾. The results of Fig. 1 demonstrate increases in several mRNAs of the Jun family, but the translation of these mRNAs remains to be examined under the present experimental conditions. In any case, these observations establish that diverse changes in gene expression must occur after insults that induce tolerance. Further studies are necessary to identify more precisely the responses necessary for ischemic tolerance, and to establish the functional significance of the expression of hsp72 and other induced proteins after ischemia.

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