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Research Report

Selective vulnerability of hippocampal sub-fields to oxygen–glucose deprivation is a function of animal age

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ABSTRACT

For more than a century, the hippocampal sub-fields have been recognized as being differentially vulnerable to injury. While the cause remains unknown, the explanations generally considered have involved either vascular differences, or innate variability among cells. To examine the latter possibility, we prepared acute hippocampal slices from Sprague-Dawley rats, applied a brief period of oxygen–glucose deprivation (OGD; an in vitro model of ischemia), and assessed the viability of dissected sub-fields (CA1, CA3, dentate gyrus) by measuring mitochondrial 2,3,5-triphenyltetrazolium chloride (TTC) metabolism. In slices from young animals (15 weeks of age), post-OGD TTC metabolism was significantly reduced in the CA sub-fields relative to the dentate gyrus. Since previous studies found increasing age may worsen ischemic injury, we completed the same experiment using tissue from animals at 52 weeks of age, and found no differences in TTC metabolism across sub-fields. Given the established role of glutamate receptors in ischemic cell death, we examined two key subunit proteins (GluN1, found in all NMDA receptors, and GluA2, found in most AMPA receptors) across sub-fields and age to determine whether their expression complemented our viability data. We found that, relative to the CA1, the DG displayed greater GluN1 expression and lower GluA2 expression in both young and old animals. Our results confirm that regional vulnerability can be shown in a slice model, that the property is not intransigent, and that these features are likely not attributable to the expression pattern of key glutamate receptor subunits, but another molecular variable that changes over the lifespan.

Abbreviations: ACSF, artificial cerebrospinal fluid; BSA, bovine serum albumin; CA, cornu ammonis; CI, confidence interval; DG, dentate gyrus; DMSO, dimethyl sulphoxide; HRP, horseradish peroxidase; LDH, lactate dehydrogenase; OGD, oxygen–glucose deprivation; PVDF, polyvinylidene fluoride; SEM, standard error of the mean; TBST, tris-buffered saline with tween-20

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1. Introduction

Although the entire brain may be inadequately perfused during global ischemia, the pattern of injury that develops is not homogeneous (Pulsinelli et al., 1982). One brain region that has received considerable attention for its selective vulnerability to ischemic injury is the hippocampus, which has also been found to display damage in a heterogeneous fashion (Schmidt-Kastner and Freund, 1991). Histological analyses of hippocampal damage following models of global ischemia in both gerbils (Kirino, 1982) and rats (Pulsinelli et al., 1982; Smith et al., 1986; Schmidt-Kastner, Hossman, 1988; Kadar et al., 1998) have revealed a relatively consistent pattern: the *cornu Ammonis* 1 (CA1) sub-field appears to be the most vulnerable region, while the dentate gyrus (DG) appears to be the most resistant region.

Although the unique susceptibility of certain areas of the hippocampus to injury was first described in the late nineteenth century (Sommer, 1880), the cause of the selective vulnerability remained a heavily debated topic for decades. The majority of discussion initially focused upon whether variation in the hippocampal vasculature might be responsible for the regional differences in injury (Spielmeyer, 1927), and then gradually gave way to the view that intrinsic differences in physicochemical properties of neurons within the hippocampus were responsible (Vogt and Vogt, 1937). Over time, the idea that the differences were attributable to innate variability in the cells comprising the hippocampus (that is, the notion of pathoclisis) began to prevail, in part due to experiments conducted with brain slice preparations.

In hippocampal slices acutely prepared from adult rats, field potentials recorded from the CA1 sub-field have been consistently abolished by a length of hypoxic insult that had a less pronounced effect upon responses evoked from the CA3 sub-field (Cherubini et al., 1989) and no permanent effect upon responses evoked from the DG (Aitken and Schiff, 1986; Kass and Lipton, 1986). As well, relative to other sub-fields, the CA1 region in hippocampal slice cultures has exhibited the greatest degree of propidium iodide uptake (a commonly used assay of plasma membrane integrity that is taken as a measure of cellular viability) following a number of insults that reflect various elements of ischemic injury, including oxygen–glucose deprivation (Gee et al., 2006), NMDA-mediated excitotoxicity (Bonner et al., 2010; Butler et al., 2010; Stanika et al., 2010), proteasomal inhibition (Bonner et al., 2010), and chemically-induced superoxide generation (Wilde et al., 1997).

Along with regional differences in the response to insult, a further general characteristic of ischemic injury that has been suggested by a number of studies is the relationship between animal age and the degree of damage. For example, the magnitude of insult following focal ischemia (as determined by infarct size, histological analysis, or neurological deficit) was consistently greater in both aged rats (Futrell et al., 1991; Yao et al., 1991; Sutherland et al., 1996; Kharlamov et al., 2000) and mice (Fuentes-Vargas et al., 2002) relative to young adult animals. As well, when hippocampal slices were exposed to either anoxia, or oxygen–glucose deprivation, tissue harvested from older rats was found to experience anoxic depolarization sooner (Roberts et al., 1990), to display a greater degree of injury-related disruption in pH regulation (Roberts and Chih, 1997), and to show a more significant degree of plasma membrane damage (Siqueira et al., 2004) relative to slices prepared from younger animals.

Given the two general trends that hippocampal tissue appears to display following ischemic injury – a tendency toward regional variation in the effect of the insult and a tendency for the degree of effect to be greater with age – the present study sought to determine how these two patterns might intersect. In particular, we wanted to examine whether differences in regional vulnerability to injury observed in younger hippocampal tissue would remain across the life span, or if there would be an increased disparity with age. To address the differences, the study employed acutely prepared hippocampal slices from rats at different ages, and then examined the effect that a period of oxygen–glucose deprivation had upon their ability to metabolize triphenyltetrazolium chloride (a commonly employed assay of mitochondrial function, Mielke et al., 2007).

2. Results

2.1. *Extracted formazan provides a sensitive, high-throughput measure for the viability of brain slices and dissected hippocampal sub-fields*

Given that TTC is metabolized within mitochondria, we considered the possibility that variation in the number of mitochondria present (that is, the amount of tissue analyzed) might influence our assay. As a result, we compared formazan values, alone and normalized to slice weight, collected from slices challenged with a moderate length of OGD (15 min). Relative to control slices (i.e., SHAM), the degree of change caused by OGD was similar regardless of whether slice weight was considered (percent of SHAM slices: formazan alone, 48.9%±3.4%; formazan relative to slice weight, 44.1%±4.3%; $n=3$ slices from each of $N=8$ animals; Fig. 1B). Since normalizing to slice weight did not seem to appreciably alter our measurement, subsequent experiments compared formazan values alone.

We next determined whether our assay would be able to detect changes in slice viability that arose as a result of increasing lengths of OGD. Slices were challenged for 5, 15, or 30 min, with the expectation that formazan levels would be reduced in a fashion inversely related to OGD length. As expected, greater lengths of OGD lead to clear reductions in TTC metabolism (formazan absorbance, arbitrary units: 5 min OGD, 0.11±0.015, $N=5$, $p=0.077$ relative to SHAM; 15 min OGD, 0.078±0.011, $N=8$, $p=0.0063$; 30 min OGD, 0.032±0.010, $N=5$, $p=0.0007$; Fig. 1C). Since a 15 min challenge provided a consistently moderate degree of reduction in formazan levels, this length of OGD was adopted as the standard for the remaining experiments.

To confirm the measure of slice viability provided by the TTC assay, which assesses mitochondrial function, we completed a set of experiments where a companion measure of viability/injury was examined. Lactate dehydrogenase (LDH) is a cytoplasmic enzyme released from cells due to injury-induced loss of cell membrane integrity, and has been readily detected in bath medium of acute hippocampal slices exposed