Neuroscience Letters 520 (2012) 20–25

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Neuroprotection supports signal processing in the hippocampus of Syrian hamsters, a facultative hibernator

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HIGHLIGHTS

- Euthermic hamster CA1 neurons are more tolerant to hypoxia than rat CA1 neurons.
- This tolerance is enhanced in neurons from hibernating versus euthermic hamsters.
- Tolerance is also greater at lower temperatures.
- Neuroprotective adaptations allow neuronal viability/function at low temperatures.
- These adaptations may contribute to survival during hibernation bouts.

ARTICLE INFO

Article history:
Received 7 March 2012
Received in revised form 10 April 2012
Accepted 2 May 2012

Keywords:
Anoxia
Hypoxia
CA1 pyramidal cells
Population spike
Evoked response
Seasonal hibernator

ABSTRACT

Studies on several species of mammalian seasonal hibernators (those hibernating only in winter) show that their neurons are more tolerant to hypoxia than those in non-hibernating species. Such tolerance has not been studied in facultative hibernators [e.g., Syrian hamsters (*Mesocricetus auratus*)], which can hibernate at any time of year. We tested the hypotheses that, when exposed to hypoxia, hamster hippocampal pyramidal cells more effectively support signal processing than do rat hippocampal neurons and this protection is enhanced in slices from hibernating versus non-hibernating hamsters and as temperature decreases. Population spike amplitudes (PSAs) were recorded from CA1 pyramidal cells. Slices were perfused in oxygenated artificial cerebral spinal fluid (O_2ACSF) to establish a baseline. Oxygen was then replaced by nitrogen (N_2ACSF) for 15 min, followed by a 30-min recovery period in O_2ACSF. Three minutes after slices were returned to O_2ACSF, PSAs recovered to $62.4 \pm 6.8\%$ of baseline in 15 slices from 8 non-hibernating hamsters but only to $22.7 \pm 5.6\%$ in 17 slices from 5 rats. Additionally, PSA recovery was greater in slices from hibernating than non-hibernating hamsters and recovery increased as temperature decreased. These significant differences ($P \leq 0.05$) suggest Syrian hamsters are a useful model for studying naturally occurring neuroprotective mechanisms.

1. Introduction

Studies of cellular properties that protect hippocampal neurons of hibernating species from apoptosis following hypoxic or ischemic insults show evidence of adaptations not seen in non-hibernating species [10,11,13]. These studies have centered primarily on 13-lined ground squirrels, *Citellus tridecemlineatus* [13], and Arctic ground squirrels, *Spermophilus parryii* [10,11], both obligatory (seasonal) hibernating species that hibernate only during the winter. There are fewer studies on neuroprotection in facultative hibernators, species that can hibernate any time of the year when exposed to appropriate environmental conditions, and to our knowledge, there have been no functional studies of the neurons of facultative hibernators challenged with either oxygen deprivation (OD) or oxygen plus glucose deprivation (OGD).

The Syrian hamster, a facultative hibernator, has proved to be a useful model for studying several aspects of signal processing during a hibernation bout, including delineation of cellular hippocampal mechanisms underlying memory formation [2,5,17], effectiveness of neuromodulators in altering electrical activity at each phase of a hibernation bout [21], and classic studies on temperature dependence of hippocampal EEG [8]. Moreover, hamsters, like seasonal hibernators such as Arctic ground squirrels, have neural adaptations enabling them to enter and arouse from hibernation while in cold environments and neurocontrollers that operate reliably over all phases of a hibernation bout [7]. One question not yet answered is whether hamsters have neuroprotective

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http://dx.doi.org/10.1016/j.neulet.2012.05.010

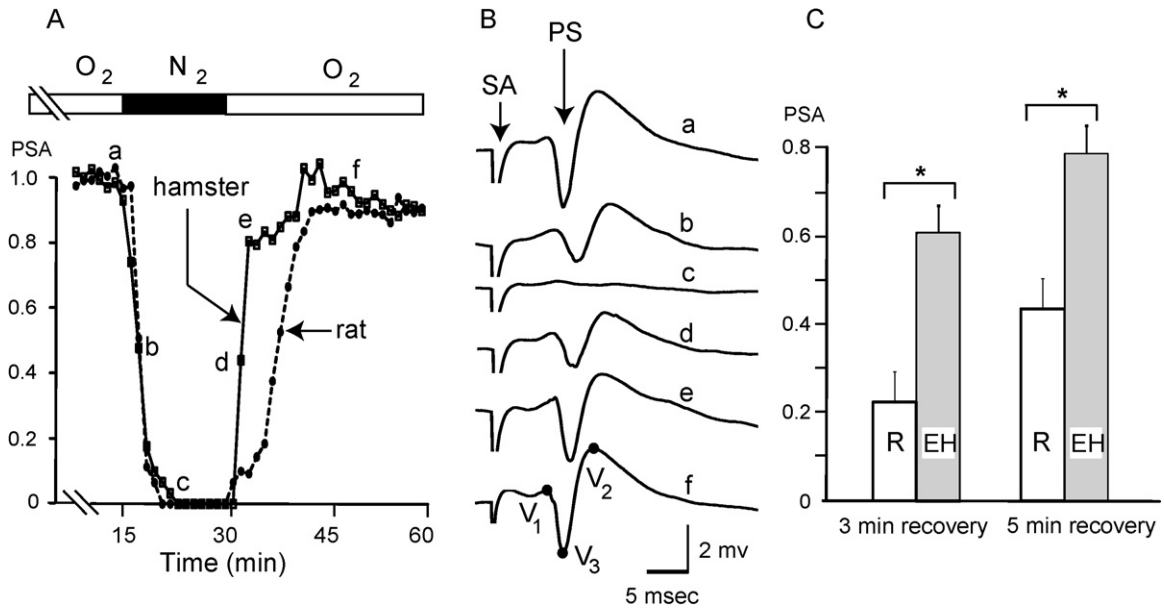

Fig. 1. Evoked responses recorded at 30 °C in hamster and rat hippocampal slices before, during, and after a hypoxic insult. (A) Comparison of population spike amplitudes (PSA) in a single rat slice versus a slice from a non-hibernating hamster. For a baseline period (left white bar labeled O_2), slices were perfused with O_2ACSF. Hypoxia was then induced by switching the solution to N_2ACSF for 15 min (black bar labeled N_2), after which the slices were returned to O_2ACSF (right white bar labeled O_2) to evaluate recovery. Normalized hamster PSAs (squares) and rat PSAs (circles) are expressed as a fraction of the PSA measured at the end of the baseline period, just prior to switching to N_2ACSF. (B) Averaged hamster evoked responses with a superimposed population spike (PS) recorded at times a–f corresponding to the sites in A with the same labels. PSA, robust at the end of the baseline period (trace a), decreased during hypoxia (traces b, c) and increased throughout the recovery period (traces d, e, f). PSA was calculated from the voltages labeled V_1, V_2, and V_3 on trace f (see Section 2). (C) Normalized group data for 17 slices from 5 rats and 15 slices from 8 euthermic hamsters show a significantly (*) more rapid PSA recovery in hamster versus rat slices (means ± standard errors; $P \leq 0.05$).

adaptations providing tolerance to hypoxia similar to those of seasonal hibernating species.

To determine if Syrian hamsters may be a useful model for studying mechanisms that provide neuroprotection against hypoxia, we tested the following hypotheses: (1) CA1 pyramidal cells in euthermic Syrian hamsters recover more rapidly after a 15 min hypoxic exposure than do such cells from rats, a non-hibernating species; (2) protection is further enhanced in hibernating versus non-hibernating hamsters; and (3) lowered brain temperature augments neuroprotection in both hibernating and non-hibernating hamsters. We also tested whether OGD impairs signal processing to a greater degree than does OD alone.

2. Methods

2.1. Brain slice preparation

Experimental protocols were approved by the UC Davis Animal Care and Use Committee in compliance with Animal Welfare Act and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Syrian hamsters (*Mesocricetus auratus*), 100–500 days old, from our hamster colony were housed at 22 ± 2 °C on a 14:10 light:dark (LD) cycle and provided with food and water ad libitum. Hamsters housed under these "summer like" conditions were non-hibernating and are designated throughout as euthermic, EH, as the animals' core and brain temperatures were ~37 °C. A subgroup of these hamsters was acclimated to a short photoperiod (8:16 LD) for four to six weeks at 22 ± 2 °C and then transferred to a 6 ± 1 °C cold room also on an 8:16 LD photoperiod, during which time most entered hibernation. Those in hibernation (designated as the HH group) had core and brain temperatures near 8 °C.

Hippocampi were sectioned into 400 μm slices and incubated for 30 min in high sucrose artificial cerebral spinal fluid (ACSF) oxygenated with 95%O_2/5%CO_2 containing (mM final concentration): 62 NaCl, 2.5 KCl, 2 $CaCl_2$, 1.5 NaH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 124 sucrose, and 10 dextrose. Slices were then transferred to a recording chamber through which flowed oxygenated ASCF (O_2ACSF) containing (mM final concentration): 124 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.5 NaH_2PO_4, 1.5 $MgSO_4$, 26 $NaHCO_3$, and 10 dextrose. To impose a hypoxic insult (OD) we perfused the slices in the chamber with N_2ACSF, a solution with the same chemical composition as the O_2ACSF but continuously gassed with 95%N_2/5%CO_2. At the conclusion of this exposure, O_2ACSF was once again perfused over the slice. ACSF flow rate was maintained at ~2 mL/min before, during, and after OD, periods indicated by the white (O_2) bars before and after the black (N_2) bar in Fig. 1A. In OGD experiments, slices were perfused with N_2ACSF, which had the same chemical composition as described above except that dextrose was replaced by sucrose. All recordings of evoked responses were made at 30 °C, except for those on the effects of temperature (Fig. 3), where each slice was tested at one of three temperatures (25 °C, 30 °C or 35 °C).

2.2. Electrophysiology

Standard methods were used to measure hippocampal responses evoked by single-shock stimulation via a bipolar tungsten electrode placed in the stratum radiatum to stimulate Schaffer collateral/commissural fibers [2,5]. Evoked CA1 pyramidal cell responses were recorded with a glass microelectrode filled with 3 M NaCl and placed in the stratum pyramidale. Included in the response was a prominent negative voltage (Fig. 1B, trace a), a population spike (PS) representing the summed response of action potentials generated by a group of CA1 pyramidal cells in response to Schaffer collateral/commissural fiber stimulation [1].

On averaged waveforms (from 60 transient evoked responses to stimulation at the rate of 1 shock/s), the PS was identified as a large negative potential (V_3) between two positive peak potentials, V_1 and V_2 (Fig. 1B, trace f). PS amplitude (PSA) was