Reappraisal of Anoxic Spreading Depolarization as a Terminal Event in Brain Slices

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In the present study we explored neuronal survival during oxygen-glucose deprivation (OGD) in slices of the barrel cortex using whole-cell patch clamp recordings and concomitant extracellular recordings of local field potential (LFP), and optical intrinsic signal (OIS). To this aim we used two types of neuronal survival assessment. In the first approach, we performed continuous recordings from a single neuron starting from control conditions and then through OGD and reperfusion. In the second approach, we sampled cells using short-term patch-clamp recordings in control conditions and during reperfusion following OGD of various durations (from 1 to 60 min).

1. Variety of electrophysiological outcomes during continuous whole-cell recordings through the oxygen-glucose deprivation and reperfusion

(a, b, c) Example traces of continuous recordings from L4 neurons starting from control conditions and then through OGD of various durations and then reperfusion. The upper black traces show E_m (10 Hz low-pass filtered to remove spikes), regular upward deflections show E_m deflections evoked by depolarizing steps to monitor cell firing and R_m . Blue insets show cell responses (non-filtered signal) to depolarizing current steps. Below, the corresponding changes in membrane resistance, DC-LFP and OIS recordings from L4, and the time course of the OGD-induced border expansion from control position are shown. Example traces for three categories of cells depending on their functional outcome after OGD: (a) Survivor; (b) Cell lost during transition to outside-out, manifested as a sharp increase in R_m during reperfusion to $>1\text{G}\Omega$ values and (c) Undetermined case which showed no recovery of E_m nor ability for spike firing upon reperfusion. (d) Average change in the OGD-induced border expansion (shaded area shows SD). (e) Raster plot of outcomes (as shown on panels a-c) as a function of reperfusion delay after OGD-induced aSD. For outside-out transitions, the time points of transition to “gigaseal” are indicated by black vertical bars. (f) Histograms of the cell count in each outcome category depending on reperfusion delay after aSD. d-e: Group data from 33 L4 neurons.

2. Variety of electrophysiological outcomes from OGD using short-term whole-cell recordings

(a) Scheme of the experiment. Cells were sampled using short-term patch-clamp recordings in control conditions and during reperfusion following OGD. (b) Voltage responses (top traces) generated by current injections (bottom traces) recorded from a L4 neuron prior to OGD. The corresponding I-V plot is shown on the right, where the red line shows the range in which R_m was measured, and E_m and R_m are indicated on the right of the I-V curve. (c) Example traces obtained from a L4 neuron which survived OGD with reperfusion started 20 min after aSD. (d) L4 cell which survived OGD with reperfusion started 59 min after aSD. (e) Putative dead cell.

3. OGD survival in L4 neurons using short-term patching approach

(a) E_m of cells patched in L4 before (left) and after OGD episodes (right) as a function of the onset of reperfusion delay after aSD. Each data point corresponds to an individual cell. Open circles show data from “other” types of cells which did not fire action potentials as the main criterion for their exclusion from a pool of live neurons. The red dotted line shows no dependence of E_m in survivor neurons on the onset of reperfusion delay after aSD. (b) The corresponding proportion of live neurons among all cells patched in L4 in a given slice (each dot represents one slice). The dashed red line represents an exponential fit for the probability of finding a live neuron in L4 as a function of the onset of reperfusion delay after aSD. (c) E_m and (d) R_m values in L4 in live neurons before OGD, and live L4 neurons and “other” cells in the post-OGD epoch (boxplots show median and 1st and 3rd quartiles). Group data from 128 control and 69 post-OGD cells from 29 slices. (** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

4. Neurons in L2/3 show higher resistance to OGD than L4 neurons

(a) Voltage responses generated by current injections in a L2/3 neuron in a slice which experienced OGD for more than 1 hour, and in which reperfusion started at a delay of 59 min after aSD. Right, the corresponding I-V plot. (b) E_m values in cells patched in L2/3 before (left) and after OGD of various reperfusion delays after aSD onset (right). Each data point corresponds to an individual cell. Filled circles correspond to live L2/3 neurons, open circles correspond to “other” cells. (c) The probability of finding a live neuron in slices that have experienced long-lasting OGDs in which reperfusion started within 30-45 and 45-60 min of aSD. Group data from 11 L2/3 and 11 L4 cells.

In conclusion, the results of the present study suggest that neuronal death evoked by OGD in submerged cortical slices may span a longer time window after aSD than initially thought, and this is more in agreement with the observations made during brain ischemia in vivo. While previously approved strategies for attenuating ischemic injury in the in vitro ischemia models are mainly aimed at delaying aSD onset our findings allow in vitro models to be used to explore neuroprotective interventions that could stop the progression of neuronal damage initiated by aSD during the post-aSD period.