ATP depletion suppresses action potential firing independently of synaptic transmission

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Introduction

Information transmission requires high levels of energy to maintain ionic gradients, Ca^{2+} extrusion and vesicular recycling (Harris *et al.*, 2012). Following action potential generation and conduction there is a large metabolic cost for restoring Na^+ and K^+ gradients (Hallermann *et al.*, 2012). Hence at times of high activity and in brain regions, such as the auditory pathway, with high metabolic rates, the balance between energy consumption and maintenance of neuronal function may compromise information transmission (Kopp-Scheinpflug *et al.*, 2011). Many studies of metabolism in brain slices have used pharmacological protocols to inhibit ATP production, and observed profound changes in network excitability; however these studies do not differentiate between suppression of postsynaptic neuronal excitability and presynaptic transmitter release in mediating the overall excitability change.

The calyx of Held/MNTB synapse in the auditory brainstem, transmits information at high frequencies and has a high metabolic rate. This is an ideal model to differentiate between presynaptic and postsynaptic metabolism at the level of a single synaptic connection.

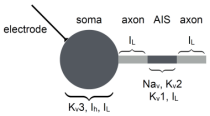
Methods

Action potential firing was studied in auditory brainstem slices from P13-P18 CBA mice using whole-cell recordings from MNTB neurons. Slices (300 µm) were placed in a recording chamber at 34 ºC and continually perfused with oxygenated artificial cerebro-spinal fluid composed of (in mM) NaCl (125), KCl (2.5), $NaHCO_3$ (26), NaH_2PO_4 (1.25), D-glucose (10), myo-inositol (3), Na- pyruvate (2), ascorbic acid (0.5), $MgCl_2$ (1), and $CaCl_2$ (2). Recording pipettes with a resistance of 3–6 MΩ were filled with a solution containing (in mM) K-gluconate (97.5), KCl (32.5), HEPES (5), EGTA (5), $MgCl_2$ (1), and NaCl (5). Current-clamp recordings were performed, with action potential firing induced by 200ms current steps of increasing amplitude.

For synaptic recording a bipolar stimulating electrode was placed at the midline to stimulate the calyceal input and MNTB neurons were voltage-clamped at -40 mV.

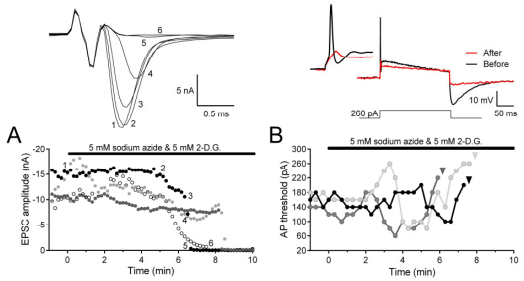
A conductance-based model was implemented using the NEURON simulation environment (Hines and Carnevale, 1997).

The ion channel models are described in Johnston *et al.* (2008). Conductance values for these ion channels were fit to data from voltage-clamp and current-clamp experiments using a multi-objective optimisation approach (Druckmann *et al.*, 2007).



ATP depletion was simulated in the model by decreasing the intracellular K^+ concentration, and/or increasing the Na^+ reversal potential.

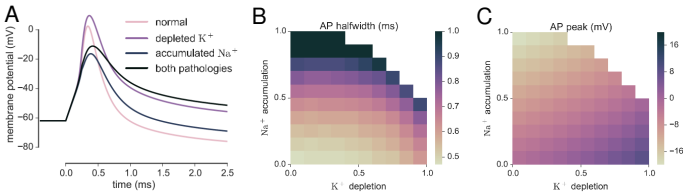
Chemical hypoxia inhibits action potential firing



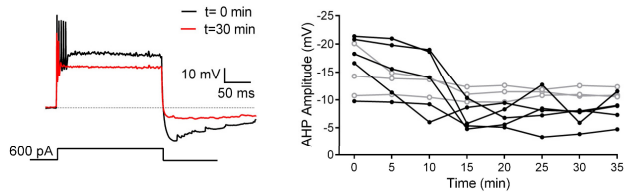
Inhibition of action potential firing by sodium azide, a mitochondrial inhibitor, and 2-deoxy-D-glucose, a glycolysis inhibitor, which are commonly used to induce a chemical hypoxia

A) 5 mM Sodium azide and 5 mM 2-deoxy-D-glucose inhibit low frequency (0.1 Hz) synaptic transmission, with a sudden loss of EPSCs in 3 out of 4 cells. B) This ATP depletion protocol results in a loss of postsynaptic action potential firing over a similar time course to the loss of EPSCs.

The inhibition of the Na^+/K^+ ATPase by ATP depletion is predicted to alter the shape of the AP waveform



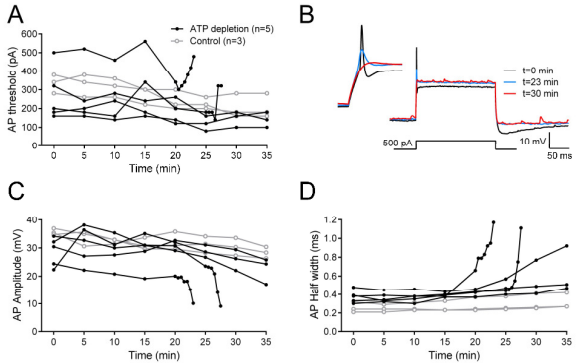
Blocking the Na^+/K^+-ATPase, to mimic ATP depletion, reduces the action potential amplitude and increases the half-width. A) The effects of only K^+ depletion and Na^+ accumulation, on simulated APs, are illustrated separately as well as the effect of ATP depletion (i.e both of these). Stimulus is a square current pulse of 1500 pA for 25 ms, starting at 0 ms. K^+ depletion and Na^+ accumulation both contribute to an increase in AP half-width (B), but affect the AP peak in opposite directions (C). Stimulus is a square current pulse of 450 pA for 200 ms. Areas filled in white correspond to values where no AP occurs.



ATP depletion, by removal of the energy substrates, glucose and pyruvate, from the aCSF and the addition of 2-deoxy-D-glucose inhibits the Na^+/K^+ ATPase mediated after-hyperpolarisation.

The Na^+/K^+ ATPase mediated after-hyperpolarisation (measured following a 600 pA step) in MNTB neurons is consistently reduced.

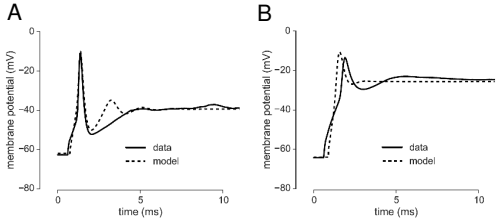
When ATP is depletion AP amplitude decreases and halfwidth increases immediately preceding AP failure



Removal of the energy substrates, glucose and pyruvate, from the perfusing aCSF and the addition of 2-deoxy-D-glucose, to deplete ATP, impairs action potential firing in some neurons.

A) Action potential threshold with ATP depletion shows that action potential failures occur in 2 out of 5 cells, with only small changes in threshold. B) Example traces from a cell where action potential failure occurs. The loss of action potential firing is preceded by a decrease in action potential amplitude (C) and an increase in half-width (D).

Fitting the MNTB model to a neuron before and after ATP depletion



Fitting the MNTB model to an intracellular recording of the action potential waveform before and after ATP depletion in the same neuron. A) Before ATP depletion using the measured capacitance of 0.63 µF cm^{-2}. B) After ATP depletion, the same conductances that achieved the fit in (A) were used but $[K^+]_{in}$ is reduced from 148 mM to 40 mM, and E_{Na} is reduced from 55 mV to 5mV. Stimulus in the experiments and model is a 200ms, 480 pA current pulse.

Conclusions

- Chemical hypoxia, generated by sodium azide with 2-deoxy-D-glucose, inhibits postsynaptic action potential firing. The loss of synaptic transmission appears to be a failure in presynaptic action potential conduction rather than a primary failure of transmitter release. This has important implications for studies using neuronal networks, since reduced activity will reflect suppression of conduction as well as transmitter release.
- ATP was depleted in the MNTB neuronal somata following removal of metabolic substrates from the perfusing aCSF and the addition of 5 mM 2-deoxy-D-glucose, resulting in a loss of the after-hyperpolarisation, which is mediated by Na^+/K^+-ATPase.
- The metabolic status of individual neurons is not the same, with some more vulnerable to ATP depletion than others; 2 out of 5 cells lost action potential firing within 35 min. The loss of firing was preceded by a decrease in AP amplitude and an increase in AP halfwidth, but there was no clear change in threshold.
- In a mathematical model of a MNTB neuron metabolism, blocking the Na^+/K^+ ATPase mimicked the effect of experimental ATP depletion reducing the action potential amplitude and increasing AP half-width.
- The Na^+/K^+ ATPase activity is reduced in all cells, but there is only a sudden change in AP waveform immediately preceding AP failure in 2 out of 5 cells, suggesting that Na^+/K^+ ATPase failure alone is not responsible for the change in AP waveform and that an additional effect, such as a change in Na^+ channel phosphorylation state maybe involved.

References & acknowledgements

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