Effects of insulin on excitability of CA1 pyramidal neurons in the rat hippocampus

E. L. Underwood, G. E. Farmer Jr. & L. T. Thompson

Aging & Memory Research Lab., School of Behavioral & Brain Sciences, Univ. of Texas at Dallas, Richardson, TX

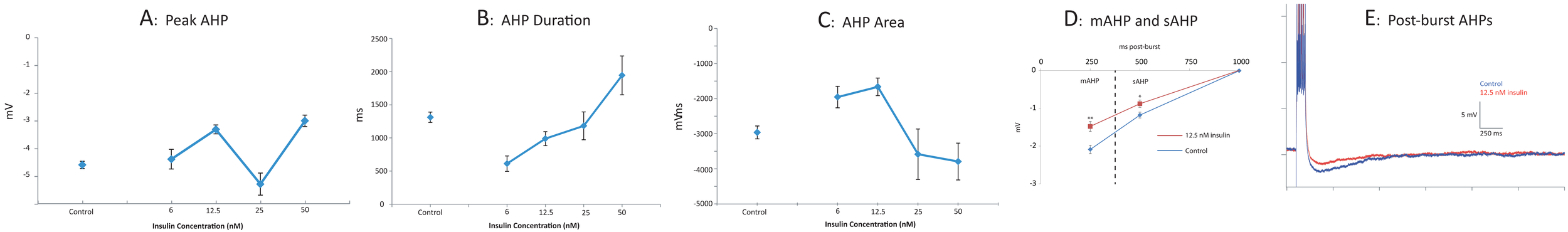


Figure 1. Insulin reduced post-burst AHPs in a concentration-dependent manner. Significant reductions were seen in: **A.** peak amplitude, **B.** duration, **C.** integrated area, and **D.** both medium (mAHP) and slow (sAHP) components of the post-burst AHP. **E.** Overlays are shown for post-burst AHPs recorded before and after bath application of 12.5 nM insulin. In early experiments post-insulin recordings were also attempted, but even with 1 hr rinse incomplete clearance of the insulin from the slice was achieved.

Background

Insulin has been shown to affect not only glucose utilization but also synaptic transmission in the CA1 region of the hippocampus, a region critically involved in learning new tasks and in consolidation of declarative memories. Learning-dependent plasticity of CA1 post-burst AHPs and spike-frequency accommodation have been reported by our own lab and many others following task acquisition, in multiple tasks and multiple species. Preliminary work from our lab has shown that insulin increases excitability of CA1 pyramidal neurons. The current study assessed effects of insulin across a range of physiological concentrations on the excitability of neurons from young adult rats with normal glycemic control. These studies are preliminary to combined behavioral (learning and memory) and neurophysiological assessment of insulin effects in rat models of diabetes.

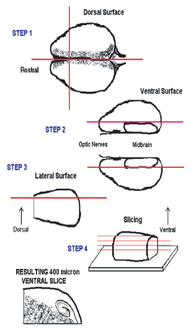
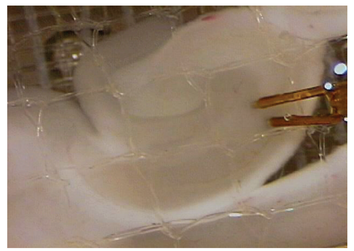


Figure 2: Ventral brain slice preparation

Figure 3: CA1 region of hippocampus



Methods

Subjects: Experiments were performed using a total of 18 naive Long Evans male rats (2-4 mo). Rats were commercially obtained from Harlan (Indianapolis, IN), and maintained in our animal facility under conditions approved by the UT Dallas ACUC on a 12hr/12hr light/dark schedule with *ad libitum* access to food and water.

Slice preparation: Rats were anesthetized with isoflurane and decapitated. The brain was quickly hemisected and immersed in cooled s-aCSF [in mM: 124 sucrose; 3 KCl; 1.3 $MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 d-glucose, pH 7.4]. After the brain chilled for 3-4 min, it was blocked and 400 µm slices cut using vibratomes then placed in room temperature (25°C) aCSF [in mM, 124 NaCl; 3 KCl; 1.3 $MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 d-glucose, pH 7.4]. Both aCSFs were continuously oxygenated (95% O_2: 5% CO_2).

Current-clamp recordings: Sharp electrodes were prepared from borosilicate glass filled with 3 M KCl (30-80 MΩ), and intracellular recordings made (using an AxoClamp-2B amplifier and National Instrument LabView interface) from submerged slices (31°C) using the protocol illustrated below. Measures of excitability (including AHP peak amplitude, duration, and area, as well as measurements of AHP amplitude at varying intervals post-burst (to assess mAHP and sAHP components) and of spike-frequency accommodation), input resistance, and hyperpolarizing sag were assessed from neurons held at -67 ± 3 mV.

Insulin Perfusion: After baseline recordings were taken in normal aCSF perfusion, a second perfusion of insulin (Lilly) treated aCSF was initiated. Insulin was allowed to perfuse the slice for a minimum of 8 min before the recording protocol was repeated. The insulin concentrations tested were 0 (control), 6 nM, 12.5 nM, 25 nM, and 50 nM. The experimenter was blind as to the insulin concentration tested during recording and data analysis.

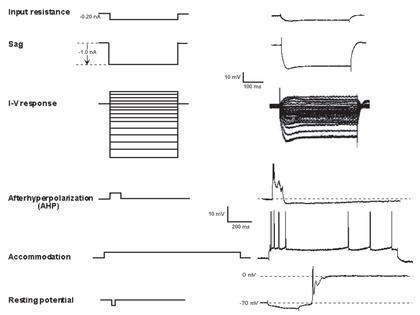


Figure 4: Intracellular (sharp-electrode) recording protocol

Results

Insulin perfusion resulted in concentration-dependent increases in excitability in CA1 pyramidal neurons. A concentration of 12.5 nM insulin produced the most consistent reductions in all measures taken. Post-burst AHPs were reduced, with significant reductions in peak amplitude, duration, integrated area, and in both mAHP and sAHP components (Figure 1 above). Accommodation (increased spike firing to a sustained input) was also reduced concentration-dependently (data not shown). The dose-dependent enhancement of hippocampal excitability observed *in vitro* was consistent with that of nootropic agents we have tested which facilitate acquisition and consolidation in declarative memory tasks in rats and rabbits.

Discussion and future directions

- These results have implications future learning and memory studies.
- These results have implications for diabetics managing blood glucose levels with insulin treatments.
- Planned future experiments include altering insulin sensitivity in rat model of diabetes and observing changes in excitability measures before and after dose-dependent insulin treatment.
- Planned future experiments will assess retention of a single-trial inhibitory avoidance learning task, will allow for comparisons between learning-induced changes in excitability and nootropic effects of insulin.
- Assessing effects of insulin on the hippocampus of aged rats should provide insight into the relationship between insulin sensitivity, type II diabetes, and age-dependent learning and memory deficits.

Acknowledgements

The study was funded by the Clark Foundation. We thank Ermias Sori for his assistance, and C. de Wolf for early ideas and preliminary data re. the effects of insulin upon hippocampal excitability.