Insulin modulates electrical activity of subfornical organ neurons

Suman Lakhi **[1]*****, Wanda Snow*** **[2]*****, and Mark Fry*** **[1]**

[1]Department of Biological Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2

[2]Department of Psychology, University of Manitoba, Winnipeg MB, R3T 2N2

Introduction

The hormone insulin is considered a key adipostat for long-term regulation of energy balance by the central nervous system (CNS). Insulin crosses the blood brain barrier (BBB) via a saturable transporter (Banks, 2004) where its main sites of action are thought to be the arcuate nucleus (ARC) and ventromedial nucleus (VMN) of the hypothalamus (Plum *et al.,* 2006). However the requirement for receptor-mediated transport across the BBB suggests that hypothalamic neurons may potentially be slow to respond as circulating concentrations of insulin change, supporting the idea that other CNS areas also play key roles.

The sensory circumventricular organs (CVOs) of the central nervous system represent an alternative and rapid pathway for the effects of insulin. CVOs lack a BBB, meaning that CVO neurons are in direct contact with circulating hormones including insulin. In particular, the subfornical organ (SFO), a forebrain CVO, is now regarded as an important centre for the regulation of energy homeostasis. For example, SFO neurons respond to many circulating satiety signals such as adiponectin, amylin, ghrelin, glucose, leptin (reviewed in Hoyda et al., 2009). Moreover, receptors for numerous satiety signals, including insulin and those mentioned above are strongly expressed in SFO (Hindmarch *et al.,* 2008) suggesting insulin pays a role in signaling at the SFO.

Objective

To determine if insulin modulates the electrical activity of subfornical organ neurons

Methods

Cell Culture

•Male Sprague-Dawley rats were decapitated, 300 µm forebrain sections cut in cold aCSF, and SFO was microdissected out.

•SFO were incubated in 2 mg/ml papain for 30 min, and gently triturated to liberate single cells. Cells were plated on 35 mm culture dishes in B27- supplemented Neurobasal media.

Patch Clamp Electrophysiology

•External recording solution consisted of (in mM) 140 NaCl, 4KCl, 10 HEPES, 10 glucose, 1 $MgCl_2$, 2 $CaCl_2$, pH 7.3.

•Electrodes had a resistance of 2-5 MΩ when filled with internal recording solution (in mM): 130 K-gluc, 10 KCl, 10 HEPES, 10 EGTA, 0.1 CaCl2, 1 MgCl2, 4 NaATP, pH 7.3.

•Insulin (Sigma) was reconstituted at 100 nM in recording saline.

•Cells that depolarized or hyperpolarized by more than 4.5 mV were considered responsive to insulin. In some experiments 3mM CsCl or 30 µM glibenclamide was added to the external solution.

Statistical Analysis

Changes in membrane potential, action potential firing rates and input resistances were compared using the Kruskal Wallace non-parametric ANOVA followed by Mann-Whitney non-parametric multiple pair-wise comparisons with Bonferroni correction. For all analyses, statistical significance was determined at the p <0.05 level.

Literature cited

Banks, WA. (2004) Eur J Pharmacol 490:5-12.
Plum L., et al. (2006) J Clin Invest 116:1886-901.
Figlewicz D., et al. (2007) Physiol Behav. 91:473-8.
Hoyda T., et al. (2009) Int J Obes 331:S16-21.
Hindmarch C., et al. (2008) Am J Physiol Regul Integr Comp Physiol.95:R1914-20.

Results

1. Insulin influences electrical properties of SFO neurons

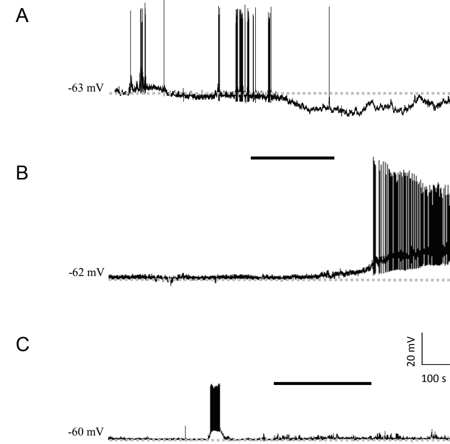

Figure 1: Insulin modulates the electrical activity of SFO neurons. Representative current clamp recordings summarizing the responses observed in SFO neurons after application of 100 nM insulin (black bar) hyperpolarization (A), depolarization (B) or insensitive (C).

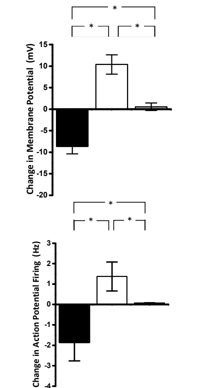

Figure 2 Insulin effects the membrane potential of SFO neurons. Bar graph, depicting mean changes in membrane potential.Black bars denote hyperpolarized; white bars denote depolarized; grey bars denote insensitive neurons. The means changes in membrane potential and action potential firing frequency were significantly different between all groups using the Mann-Whitney non parametric pairwise comparison test with a Bonferroni correction. * p < 0.05

Figure 3 Insulin effects the action potential firing frequency of SFO neurons. Bar graph, depicting mean changes in action potential firing**.** Black bars denote hyperpolarized; white bars denote depolarized; grey bars denote insensitive neurons. The means changes in membrane potential and action potential firing frequency were significantly different between all groups using the Mann-Whitney non parametric pairwise comparison test with a Bonferroni correction. * p < 0.05

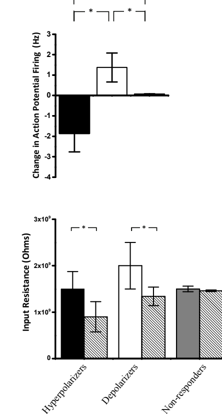

Figure 4: Insulin differentially affects the input resistance of SFO neurons. Bar graph demonstrating the mean changes in input resistance following insulin application. Solid bars represent input resistance before application of 100 nM insulin, hatched bars represent after. Black bar indicates cells whose membrane potential hyperpolarized, white bar indicates cells whose membrane potential depolarized and grey bar indicate cells whose membrane potential did not change. * p < 0.05

2. Insulin modulates K_{ATP} and I_h channels in SFO neurons

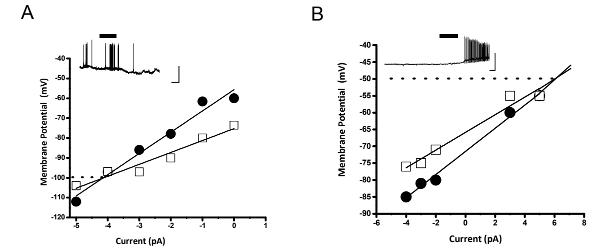

Figure 5 (V/I) plots showing the change in input resistance after application of 100 nM insulin. Circles represent before application of insulin, squares represent after insulin. (A) Crossover point of the two lines in neurons that hyperpolarized is near -100 mV, indicating activation of K^+ channels. (B) Crossover point of the two lines in neurons that depolarized is near -55 mV, indicating activation of cation channels such as non-specific cation channels.

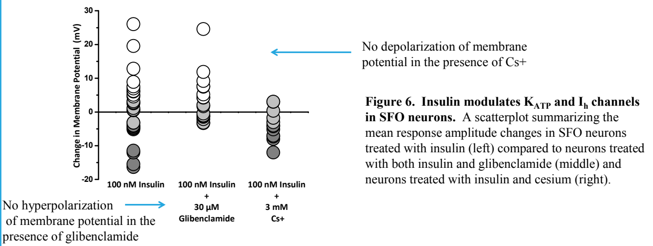

Figure 6. Insulin modulates K_{ATP} and I_h channels in SFO neurons. A scatterplot summarizing the mean response amplitude changes in SFO neurons treated with insulin (left) compared to neurons treated with both insulin and glibenclamide (middle) and neurons treated with insulin and cesium (right).

3. Identity of ion channels modulated

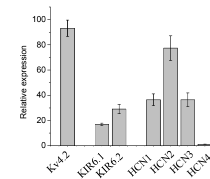

Figure 7. Hindmarch *et al.*, (2008) carried out a gene array experiment to quantify gene expression in the subfornical organ. The bar graph to the right identifies the potential ion channels targeted by insulin. Kv4.2 id included for reference.

Summary

•This study demonstrates for the first time that insulin modulates the electrical activity of SFO neurons and provides support for the notion that the SFO as an important sensor for regulating energy homeostasis.

•Moreover, this work provides a clear understanding of ionic mechanisms targeted by insulin for the regulation of electrical activity in SFO neurons. While insulin has been demonstrated to regulate KATP in hypothalamic centres, this is one of the first descriptions of an insulin-mediated depolarization.

Support