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Hippocampal Extracellular Potassium Levels and Formation of Spatial Memory in Response to Retrodialysis Insulin Administration

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Abstract

Insulin is the most common treatment for hyperglycemia, such as that caused by type 1 or type 2 diabetes mellitus. Insulin causes cellular uptake and storage of glucose to maintain homeostasis and also plays important roles in other systems; an important example is regulation of potassium. In the periphery, insulin administration has been shown to increase the cellular uptake of potassium via Na^+/K^+ ATPase, leading to hypokalemia. Research in our lab and others has shown that insulin is a key regulator of cognitive function and local metabolism within the hippocampus. To date, however, no studies have examined whether insulin acts to regulate potassium levels within the brain, nor whether such regulation might correlate with cognitive effects caused by administration of exogenous insulin.

The current study sought to extend our previous work on the impact of exogenous insulin delivery to the hippocampus: specifically, we measured the impact of intrahippocampal insulin administration on local extracellular potassium levels, both at baseline and during a cognitive task. Rats were tested using a spontaneous alternation task in a four-arm maze with concurrent microdialysis and retrodialysis of insulin for the period corresponding to the behavioral task. Insulin was added to the perfusate at 10 µl in an artificial extracellular fluid vehicle. Samples were collected throughout acclimation, baseline, testing, and recovery periods, and will be analyzed for potassium and glucose. Hippocampi and prefrontal cortices were collected and will be analyzed for Na^+/K^+ ATPase protein concentrations. We hypothesize that insulin administration will lower extracellular potassium levels, and Na^+/K^+ ATPase will be upregulated in relation to controls. Future research should investigate the specific cognitive impacts of potassium imbalances.

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Introduction

Insulin is widely considered the most effective treatment for type 1 diabetes as well as T2DM. Even insulin analogues are not tolerated by many patients, who must use exogenous insulin to effectively treat their condition (Maria Rotella, Pala, & Mannucci, 2013). More than 100 million U.S. adults are now living with diabetes or prediabetes (CDC Newsroom, 2017). In addition to being taken as a treatment for diabetes, exogenous insulin may be taken to support wound healing activity, for body building in athletes, as an antiaging agent, as a diagnostic test to check hypothalamo-pituitary-adrenal (HPA) axis activity, to treat septic shock, or as one of the key ingredients of parenteral nutrition formulas (Benni & Patil, 2016). An important difference between exogenous and endogenous insulin is that endogenous insulin is released in proximity to the liver, where much of it is absorbed and less than 50% continues on to peripheral tissues. Exogenous insulin first circulates through the peripheral tissues before arriving at the liver, meaning peripheral hyperinsulinemia must occur in order for there to be an adequate amount to regulate the liver (Lebovitz, 2011).

It is common for diabetic patients using exogenous insulin to develop electrolyte imbalances, including hypokalemia in the peripheral bloodstream (Liamis, Liberopoulos, Barkas, & Elisaf, 2014). Exogenous insulin causes peripheral hypokalemia by increasing the entry of potassium (K^+) into skeletal muscles and hepatic cells. It does this by stimulating the activity of Na^+-K^+-ATPase pumps (Liamis et al., 2014).

One possible mechanism through which insulin stimulates Na^+/K^+-ATPase activity is reversible modification of catalytic subunits of the pump. Another is modification of the gene or protein expression for a pump subunit (Sweeney & Klip, 1998). An additional, highly debated mechanism is the translocation of Na^+-K^+-ATPase to the surface of skeletal muscle cells to

manipulate pump abundance; this phenomenon has been observed by some researchers but not others (Benziane & Chibalin, 2008).

Cells of the central nervous system also express Na^+/K^+-ATPase pumps. These pumps are crucial for neuronal excitability and healthy brain function (Scherer et al., 2009). Neurons and glial cells express different isoforms of the pump, and some neurons express multiple isoforms to ensure that proper intra- and extra-cellular levels of potassium are maintained (Dobretsov & Stimers, 2005).

Research has shown that insulin, produced mostly by the pancreas, crosses from the periphery into the CNS through the blood-brain barrier via a saturable transport system (Banks, Owen, & Erickson, 2012). Further research recorded the time it took radioactive insulin to travel from plasma to the cisternal CSF in dogs; the results supported the hypothesis that there is a transitional "compartment" between peripheral plasma and cisternal CSF (Schwartz et al., 1991). This research shows that hyperinsulinemia in the periphery due to administration of exogenous insulin could cause hyperinsulinemic conditions in the brain.

In our research, we examined whether the hypokalemia that occurs in the periphery in response to exogenous insulin is also occurring in the central nervous system through the action of insulin on the Na^+/K^+-ATPase pumps expressed by neurons and glial cells. Healthy potassium levels are a cornerstone of normal nerve function. Active transport of potassium into the cell causes depolarization, and the original levels of potassium must be restored before the next impulse can be generated. Even a decrease in extracellular potassium levels by as little as 1% changes the electrophysiology of the cell membrane and may impair impulse generation, and therefore cognitive function (Kowey, 2002). With such a high prevalence of diabetes, and such a large quantity of diabetic patients using insulin therapy to manage blood sugar levels – as well as

a variety of other populations using exogenous insulin for other purposes – this is crucial information when considering the use of exogenous insulin as a treatment option.

We used microdialysis and spontaneous alternation testing in a rat model to address this question. Microdialysis allowed us to administer exogenous insulin directly to the hippocampus at a controlled dosage and take samples of the surrounding extracellular fluid to check potassium levels following insulin administration, thereby exploring the question of whether or not hypokalemia occurs in the CNS in response to exogenous insulin. Spontaneous alternation allowed us to evaluate the cognition of the rats as a function of brain potassium levels and explore the question of how exogenous insulin modulates cognitive function.

Methods

20 Sprague Dawley (Crl: CD (SD), Charles River, Kingston, NY), age p77 at arrival, were split into two groups. All animals underwent the same timeline: Arrival day 0, handling days 2-8, surgery day 9, recovery days 10-16, microdialysis and spontaneous alternation day 17 followed immediately by euthanasia. Surgery used standard IACUC-approved surgical technique and accomplished microdialysis cannulation of the left hippocampus.

Testing: Each animal had a clean probe briefly placed into the cannula 24 hours before behavioral testing. Protocols used in previous research also included this step (Adell & Artigas, 1998; Zapata, Capdevila, & Trollas, 1998; Harte & O'Connor, 2005). The diameter of the probes is larger than size of, and spacing between, the cells and vessels in the brain. When inserted, a probe causes ischemia, gliosis, and cell death at the site and activates the brain's astrocytes. The astrocytes respond by engulfing the probe, ultimately forming a glial scar around it that appears clearly after 5 days (Nesbitt, Jaquins-Gerstl, Skoda, Wipf, & Michael, 2013). 24 hours after insertion, the site has had time restore neurochemicals to levels closer to normal but has not had time to form disruptive scar tissue around the probe.

A fresh probe (CMA12 elite, 20kDA cutoff 4mm length) was placed into the cannula at the beginning of the microdialysis procedure. The rat had a 60-minute acclimation period after probe insertion. Samples were collected into pre-weighed, pre-labelled 0.6mL microtubes. Each sample corresponded to one of nine time periods: 1 acclimation (time (t)=1 hour), 3 baselines (t=20 min), 2 testing (t=10 min), and 3 recovery (t=20 min). The perfusate was artificial extracellular fluid containing 2% bovine serum albumin (BSA). BSA, which is too large to pass through the probe's membrane and stays in the tubing, effectively reduces loss of analyte

molecules in the perfusate that stick to the sides of the tubing (Kendrick, De La Riva, Hinton, & Baldwin, 1989).

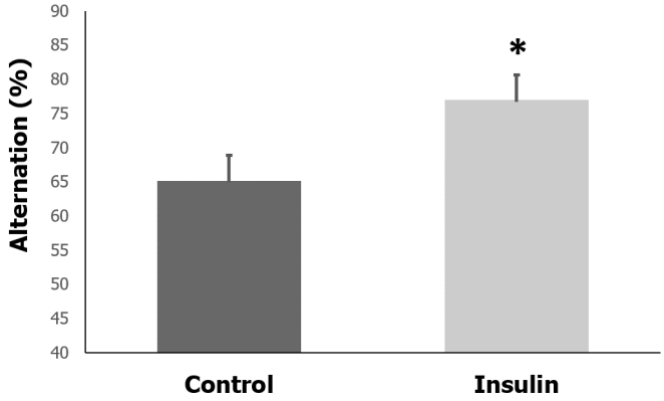
The perfusate was run through the tubing the night before the procedure to minimize insulin loss to the FEP tubing. The experimental group received insulin in the perfusate at 10 µU/min for duration of the 20-minute testing period; insulin administration was controlled by switching the source of the perfusate to a second syringe containing insulin for 20 minutes. The switch was made at a time calculated such that insulin arrived at the rat as the spontaneous alternation testing began.

Histology: At euthanasia, each animal was weighed before anesthesia induction, then decapitated. Whole blood samples were collected, and serum was separated out. The hippocampi were isolated and frozen separately. Microdialysate will be tested for potassium levels using a potassium assay (Crystal Chem, catalog # 80169).

Results

Group 1 received insulin but did not undergo behavioral testing. Tissue samples from group 1 will be tested for potassium levels. Insulin enhanced rats' performance on spontaneous alternation (SA) testing (Fig. 1).

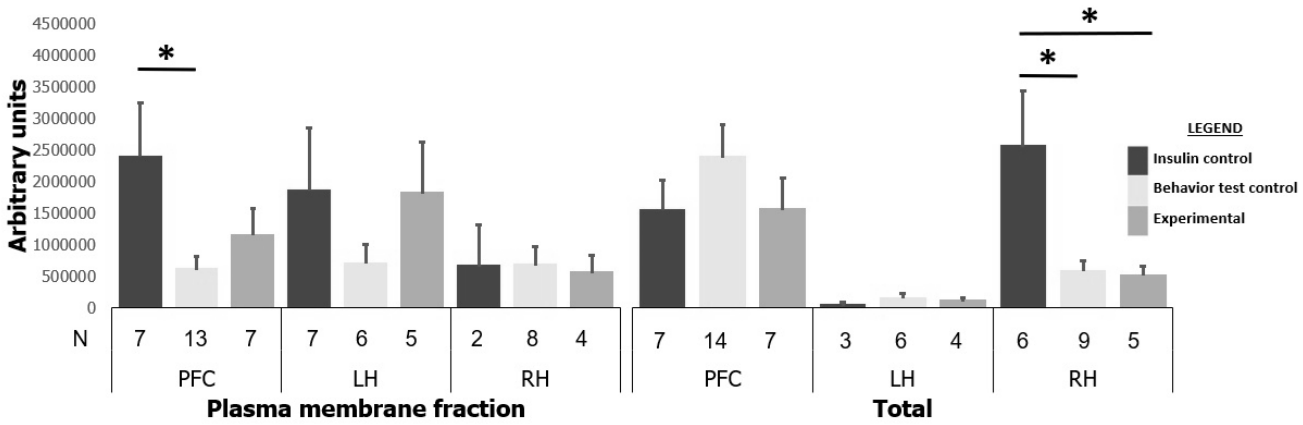
Fig. 1: Effect of insulin administration on spatial memory. *denotes $p<0.05$. Error bars represent the standard error. N=8/group



Group 2, which did not receive insulin, had an average SA score of 65.18% (n=8) with a standard error of 3.69%. Group 3, which received insulin, had an average SA score of 76.98% (n=8) with a standard error of 3.67%. This is consistent with previous research in which control-group rats had an average SA score of approximately 60%, while rats who received 100 μU of intrahippocampal insulin had an average SA score of approximately 74% (McNay et al., 2010).

Insulin administration was also associated with increased levels of plasma membrane bound Na+/K+ ATP-ase in the right hippocampus and in the prefrontal cortex (Fig. 2).

Fig. 2: Effect of insulin administration on Na+/K+ ATP-ase. *denotes p<0.05. (SA, no insulin; no SA, no insulin; SA and insulin). Error bars represent the standard error. PFC: prefrontal cortex. LH: Left hippocampus RH: Right hippocampus



Discussion

The unchanged levels of Na^+/K^+-ATPase in the left hippocampus represent an unexpected finding. This may be a result of Western blot errors such as inappropriate antibody volume, excessive or not enough sample, or unintended digestion of the protein in the samples before imaging. More blots need to be run in order to investigate this finding and falsify or corroborate it.

The increased levels of plasma membrane-bound Na^+/K^+-ATPase in the right hippocampus and prefrontal cortex in the presence of excess insulin are consistent with the demonstrated effects of excess insulin in the periphery.

Our finding that hippocampal insulin administration increases performance on a spontaneous alternation task is consistent with previous research. It was previously shown that insulin in the rat hippocampus enhances spatial memory and improves SA performance (McNay et al., 2010); our data replicate and confirm that finding.

More work is needed to identify the effects of insulin on brain potassium levels. The extracellular fluid surrounding the brain's cells is rich in Na^+ ions and poor in K^+ ions, while neurons themselves are poor in Na^+ ions and rich in K^+ ions. The resulting chemical gradient across the cell membrane is crucial to processes such as activation and inactivation of voltage-gated channels, synaptic transmission, and electrogenic transport of neurotransmitters (Kofuji & Newman, 2004). If insulin significantly disrupts the potassium gradient across the membrane by moving more of the ions into cells, some of these processes might be impacted and cognition impaired as a result.

However, there are diverse cellular mechanisms for tight control of potassium levels in the CNS of vertebrates and invertebrates alike. Insulin's impact on extracellular potassium levels

may be mitigated by mechanisms like potassium buffering by glial cells and have no effect on cognition (Kofuji & Newman, 2004). On the other hand, research has shown that increased availability of potassium improved cognitive performance, increased LTP generation, decreased inflammatory markers such us IL-6 and GFAP and decreased the oxidative stress marker 4-HNE (Cisternas et al., 2015). Perhaps increased availability of intracellular potassium might enhance cognitive performance.

If we are to continue using insulin for a wide variety of purposes (ie., diabetes, wound healing, body building, antiaging, checking HPA activity, septic shock, parenteral nutrition formulas [Benni & Patil, 2016]) and continue to allocate funds for researching other ways insulin can improve lives, we must understand not only its effect as a cognitive enhancer but also its effects on other physiologically-relevant molecules.

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