Glucose sensitivity of mouse olfactory bulb neurons is conveyed by a voltage-gated potassium channel

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Key points

- Potassium ion channels dampen excitability of neurons but may also be sensors of internal metabolism.
- Mice with gene-targeted deletion of the potassium channel Kv1.3, a channel regulating action potential spike frequency in the olfactory bulb, are ‘super-smellers’ and resistant to diet-induced obesity.
- Electrophysiology experiments demonstrate that Kv1.3 is sensitive to the active form of glucose and that Kv1.3-expressing mitral neurons of the olfactory bulb are predominantly inhibited by a change to high glucose concentration.
- Modulation of the neuron target properties of spike firing rather than action potential shape involves synaptic activity of glutamate or GABA signalling circuits, and is dependent upon Kv1.3 expression.
- Given the rising incidence of metabolic disorders attributed to weight gain, changes in neuronal excitability in brain regions regulating sensory perception of food are of consequence if we are to understand the function of the brain under chronic hyperglycaemia as is typical with obesity.

Abstract

The olfactory bulb has recently been proposed to serve as a metabolic sensor of internal chemistry, particularly that modified by metabolism. Because the voltage-dependent potassium channel Kv1.3 regulates a large proportion of the outward current in olfactory bulb neurons and gene-targeted deletion of the protein produces a phenotype of resistance to diet-induced obesity in mice, we hypothesized that this channel may play a role in translating energy availability into a metabolic signal. Here we explored the ability of extracellular glucose concentration to modify evoked excitability of the mitral neurons that principally regulate olfactory coding and processing of olfactory information. Using voltage-clamp electrophysiology of heterologously expressed Kv1.3 channels in HEK 293 cells, we found that Kv1.3 macroscopic currents responded to metabolically active (D-) rather than inactive (L-) glucose with a response profile that followed a bell-shaped curve. Olfactory bulb slices stimulated with varying glucose concentrations showed glucose-dependent mitral cell excitability as evaluated by current-clamp electrophysiology. While glucose could be either excitatory or inhibitory, the majority of the sampled neurons displayed a decreased firing frequency in response to elevated glucose concentration that was linked to increased latency to first spike and decreased action potential cluster length. Unlike modulation attributed to phosphorylation, glucose modulation of mitral cells was rapid, less than one minute, and was reversible within the time course of a patch recording. Moreover, we report that modulation targets properties of spike firing rather than action potential shape.
In addition, Kv1.3 participates in a cadre of non-traditional functions outside of that of cellular electrical excitability, such as cellular proliferation, axonal targeting, insulin sensitivity, apoptosis, protein expression and scaffolding as reviewed by Kaczmarek (2006).

A combination of voltage-clamp, immuno-cytochemical, and co-immunoprecipitation experiments have demonstrated that the Kv1.3 channel can be modulated by multiple tyrosine kinase signalling cascades and adaptor proteins giving rise to complex biophysical control over its many functions via the formation of molecular scaffolds (Fadool & Levitan, 1998; Cook & Fadool, 2002; Marks & Fadool, 2007; Colley et al. 2009). Specifically, receptor tyrosine kinases, such as the insulin receptor and tyrosine receptor kinase B, as well as cellular tyrosine kinases, such as src kinase, are expressed in mitral cells of the olfactory bulb and have been shown to acutely suppress Kv1.3 current by direct tyrosine phosphorylation (Bowlby et al. 1997; Fadool et al. 1997, 2000; Fadool & Levitan, 1998; Cayabyab et al. 2000; Cook & Fadool, 2002; Tucker & Fadool, 2002; Colley et al. 2004). Serine/threonine phosphorylation by protein kinase A and protein kinase C results in increases or decreases in Kv1.3 current, depending on cell type (Chung & Schlichter, 1997a,b; Martel et al. 1998). In T-lymphocytes, reactive oxygen species (ROS), byproducts of glucose metabolism, have also been shown to suppress Kv1.3 activity (Duprat et al. 1995; Cayabyab et al. 2000) while ATP, another byproduct of glucose metabolism, has been shown to increase Kv1.3 activity (Chung & Schlichter, 1997a). Acute or chronic changes in proteins that regulate or activate any of these pathways will change the electrical activity of this channel and thereby affect cellular membrane electrical properties as well as the non-traditional roles in which Kv1.3 participates.

We have focused our recent investigation of Kv1.3 on the olfactory system. Here, Kv1.3 channels contribute 60–80% of the outward voltage-gated current of mitral cells, the primary output neuron of the olfactory bulb, therefore the channel’s activity is important in the timing and duration of action potential spike trains and neuronal excitability (Fadool & Levitan, 1998; Colley et al. 2004; Fadool et al. 2011; Mast & Fadool, 2012). Gene-targeted deletion of Kv1.3 (Kv1.3−/−) results in mice that are ‘super-smellers’, possessing an enhanced ability to detect and discriminate odours (Fadool et al. 2004). Acute intranasal delivery of insulin to the olfactory bulb of wild-type (WT) mice increases tyrosine-specific phosphorylation of Kv1.3 and results in an increase in olfactory discriminatory ability (Marks et al. 2009). Insulin-dependent tyrosine-specific phosphorylation of Kv1.3 has been previously shown to suppress Kv1.3 current in mitral cells (Fadool et al. 1997, 2000; Fadool & Levitan, 1998; Colley et al. 2004). Mitral cells from Kv1.3−/− mice exhibit slowly inactivating Kv currents more typical of traditional delayed rectifiers with little or no cumulative inactivation (Fadool et al. 2004) and have recently been found to have an increase in Na+ -activated potassium currents (Lu et al. 2010). These changes in the outward current due to gene-targeted deletion of Kv1.3 result in an increased mitral cell firing frequency and decreased interspike interval (Fadool et al. 2004, 2011). Moreover, mice made obese through maintenance on a high-fat diet, display resistance to insulin-induced modulation of mitral cell firing patterns (Fadool et al. 2011).
The drive to search for and consume food is normally triggered by internal metabolic needs, signalled by changing hormones and metabolites such as insulin and glucose. The ability to find, make a qualitative judgment about, and attain food, is dependent on external sensory stimuli, such as olfactory and visual cues signalling availability of palatable nutrients in the environment. Satiation decreases and fasting increases rodent odour detection ability (Aimé et al. 2007) as well as mitral cell electrical activity in response to food odours (Pager et al. 1972). These observations imply that energy status and feeding state can modulate olfactory sensitivity. In fact, leptin, insulin, and other metabolically regulated hormones have been found to modulate, or be modulated by, olfaction as reviewed by Palouzier-Paulignan et al. (2012). However, little is known about the affect of glucose on the olfactory system even though serum glucose levels vary acutely with feeding state, with low levels after periods of fasting indicating the need to find food, making it an ideal candidate molecule for food seeking-based olfactory modulation.

In as far as Kv1.3 has previously been found to be sensitive to byproducts of glucose metabolism such as ROS and ATP (Duprat et al. 1995; Chung & Schlichter, 1997a; Cayabyab et al. 2000) and the fact that targeted deletion of the channel alters whole animal metabolism (Xu et al. 2003, 2004; Fadool et al. 2004; Tucker et al. 2008, 2012b), we hypothesized that Kv1.3 could be acting as a metabolic sensor of peripheral glucose concentration by changing mitral cell sensitivity with energy availability. Curiously, Kv1.3+/− mice are resistant to diet and genetic obesity. When challenged with a high-fat diet, they are not only resistant to adiposity, they remain normoleptinaemic and are hypoglycaemic (Tucker et al. 2008, 2012b). In fact, removal of the olfactory bulb in Kv1.3+/−, obesity-resistant mice, abolishes their resistance to weight gain due to abrogation of increased basal metabolic rate (Tucker et al. 2012a). To determine if the Kv1.3 channel was a metabolic sensor for the olfactory bulb via glucose sensing, we first studied changes in the biophysical properties of the cloned channel via voltage-clamp electrophysiology as heterologously expressed in human embryonic kidney cells (HEK 293) by exposing the isolated channel to various concentrations of metabolically active (D-) or metabolically inactive (L-) glucose isoforms. Olfactory bulb slices were then bathed in a wide range of glucose concentrations while monitoring mitral cell excitability by current-clamp electrophysiology in WT and Kv1.3−/− mice. Mitral cells from WT mice displayed both increased and decreased action potential firing frequency in response to D-glucose without modification of action potential shape, whereas, Kv1.3−/− mice completely lacked glucose sensitivity.

Methods

Ethical approval

All experiments described in this report were approved by the Florida State University Institutional Animal Care and Use Committee (IACUC) under protocol no.1124 and were conducted in accordance with the American Veterinary Medicine Association (AVMA) and the National Institutes of Health. In preparation for olfactory bulb slice electrophysiology, mice were anaesthetized with isoflurane (Aerrane; Baxter, Deerfield, IL, USA) using the IACUC-approved drop method and were then killed by decapitation (AVMA Guidelines on Euthanasia, June 2007).

Solutions and reagents

HEK 293 extracellular pipette solution contained (in mM): 30 KCl, 120 NaCl, 10 Hepes and 2 CaCl2 (pH 7.4). HEK 293 bath solution contained (in mM): 150 KCl, 10 Hepes, 1 EGTA and 0.5 MgCl2 (pH 7.4). These solutions were designed to be suitable for cell-attached recordings to stabilize the resting potential and prevent saturation of the amplifier given the robust expression of the channel (Bowley et al. 1997). HEK 293 pipette and bath solutions were supplemented with 0, 5, 10 or 20 mM D- or L-glucose, and osmotically balanced with D-mannitol. Slice intracellular pipette solution contained (in mM): 135 potassium gluconate, 10 KCl, 10 Hepes, 1 MgCl2, 0.4 NaGTP and 2 NaATP (pH 7.3; 280–285 mosmol l−1). Artificial cerebral spinal fluid (ACSF) contained (in mM): 119 NaCl, 26.2 NaHCO3, 1 NaH2PO4, 2.5 KCl, 1.3 MgCl2, 2.5 CaCl2 and 22 D-glucose (pH 7.3; 310–315 mosmol l−1). Sucrose-modified ACSF for sectioning contained (in mM): 83 NaCl, 26.2 NaHCO3, 1 NaH2PO4, 3.3 MgCl2, 0.5 CaCl2, 72 sucrose, and 22 D-glucose (pH 7.3; 315 mosmol l−1) (Saint Jan & Westbrook, 2007). Depending on the slice experiment, the D-glucose concentration of the ACSF was changed to 0, 5, 10 or 22 mM D- or L-glucose, and osmotically balanced with D-mannitol to 315 mosmol l−1. It is not atypical for slice recordings to have a slightly hyposmotic pipette solution with respect to that of the bath in order to promote seal formation and increase the longevity of its integrity, as we and others have previously reported (Carlson & Coulter, 2008; Fadool et al. 2011). All salts and sugars were purchased from Sigma-Aldrich (St Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA). Tissue culture and transfection reagents were purchased from Invitrogen (Carlsbad, CA, USA). The synaptic blockers (10 μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX), 25 μM D-amino-5-phosphonovalerate (D-APV),
and 10μM gabazine) were purchased from Ascent Scientific (Princeton, NJ, USA).

Cell line maintenance and transient transfection

HEK 293 cells were maintained in accordance with the Guidance on Good Cell Culture Practices (G CCP) as established by the European Centre for the Validation of Alternative Methods Task Force on GCCP (Coecke et al. 2005). Cells were grown in modified Eagle’s medium (MEM; Invitrogen) supplemented with 2% penicillin/streptomycin (Sigma-Aldrich) and 10% fetal bovine serum (Invitrogen) at 37°C and 5% CO2. The Kv1.3 cDNA construct was generated previously by subcloning Kv1.3 into the pcDNA3 vector (Invitrogen) at the unique HindIII site in the multiple cloning region, placing the channel-coding region downstream from a cytomegalovirus promoter (Holmes et al. 1996). cDNA encoding human CD8 was amplified from pCDM8 and subcloned into pcDNA3 between the BamH1 and EcoR1 restriction sites (Mast et al. 2010). Before transfection, cells were grown to confluence (7 days), dissociated with trypsin-EDTA (Sigma-Aldrich) and mechanical trituration, diluted in MEM to a concentration of 600 cells ml−1, and re-plated on Corning dishes (Fisher Scientific). cDNA was introduced into the HEK 293 cells with a lipofectamine reagent (Invitrogen) 3–4 days after cell passage. At the time of transfection, the cells were approximately 20–30% confluent. Lipofectamine and DNA were allowed to complex for 15 min. The DNA/lipofectamine complex was diluted in 1 ml of serum-reduced OptiMEM (Invitrogen), and cells were co-transfected for 4–4.25 h with 1μg of Kv1.3 cDNA and 0.1μg of the hCD8 construct per 35 mm dish. Co-transfection with the hCD8 construct provided a means of rapidly selecting transfected cells for electrophysiology. Before patch recording, cells were rinsed with bath solution and incubated with 1:1000 anti-hCD8 beads in bath solution (Dyna-Beads, Invitrogen) to mark transfected cells (Mast et al. 2010).

HEK 293 electrophysiology

Cells were visualized at 10× and 40× using a Zeiss Axiolab 135 microscope equipped with Hoffman optics (Carl Zeiss Microimaging Inc., Thornwood, NY, USA). Macroscopic, voltage-activated currents were generated and captured using pCLAMP9 software in conjunction with an Axopatch 200B amplifier (Axon Instruments, Molecular Devices, Sunnyvale, CA, USA) while voltage-clamping cells in a cell-attached configuration. The patch pipettes were fabricated from borosilicate electrode glass from Jencos Limited (VWR International Ltd, Leighton Buzzard, UK), fire-polished to a tip diameter of approximately 1μm (Mittman et al. 1987), and coated with beeswax near the tip to reduce pipette capacitance. HEK 293 cells that heterologously expressed Kv1.3 channels were preincubated for 30 min at 37°C in HEK 293 bath solution containing 0 mM (hypoglycaemic), 5 mM (normoglycaemic), 10 mM (fed-state), or 20 mM (hyperglycaemic) metabolically active D-glucose, or the metabolically inactive L-glucose prior to and during electrophysiological recording. D-Mannitol was used as a metabolically inactive osmotic balance for the glucose so that each test bath solution contained a 20 mM total of glucose and/or mannitol. The extracellular pipette solution contained the same glucose/mannitol balance as the bath solution during each recording. No visible signs of osmotic stress were observed prior to or during recording. Typically cells were voltage clamped to a holding potential (Vh) of −90 mV and stepped to a command voltage (Vc) of +40 mV for 1000 ms before returning to Vh. An interpulse interval of at least 60 s was used to allow for complete recovery from cumulative inactivation (Marom & Levitan, 1994). At the onset of recording, voltage-activated currents were allowed to stabilize for 3–5 min, after which, peak current magnitude and rate of inactivation and deactivation were measured (see ‘Data analysis and statistics’ section below). Families of current–voltage relations were generated by holding the cell at −90 mV and stepping from −80 to 60 mV in 20 mV increments for 1000 ms before returning to Vh. Peak current magnitude at each glucose concentration was normalized to the 0 mM glucose concentration within a transfection so that any variation in transfection efficiency did not mask the treatment effect.

Animal care and mouse lines

All mice were housed at the Florida State University vivarium in accordance with the institutional requirements for animal care. All mice used in this study (C57BL/6 background strain) were maintained on a standard 12 h/12 h light/dark cycle and were allowed ad libitum access to 5001 Purina Chow (Purina, Richmond, VA, USA) and water. Mice were of both sexes and ranged from postnatal day 21 to 35 at the time of the experiment. A total of 34 wild-type (WT) and a total of 14 Kv1.3−/− mice were used in the study. Kv1.3−/− mice were previously produced by excision of the Kv1.3 promoter region and one-third of the 5′ coding region (Koni et al. 2003; Xu et al. 2003) and were a generous gift from Drs Leonard Kaczmarek and Richard Flavel (Yale University, New Haven, CT, USA).

Olfactory bulb slice electrophysiology

The olfactory bulbs were exposed by removing the dorsal and lateral portions of the cranium between the cribriform plate and the lambda suture as described by Nickell
and colleagues (Nickell et al. 1996). After removing the dura, the olfactory bulbs (while still attached to the forebrain) were quickly removed, glued to a sectioning block with Superglue (Lowe’s Home Improvement, USA) and submerged in oxygenated, ice-cold, sucrose-modified ACSF to prepare the tissue for sectioning (Saint Jan & Westbrook, 2007). Horizontal sections (275 μm) were cut in oxygenated, ice-cold, sucrose-modified ACSF using a Series 1000 Vibratome (Vibratome/Leica,Wetzlar, Germany). The sections were allowed to recover in an interface chamber (Krimer & Goldman-Rakic, 1997) with oxygenated, sucrose-modified ACSF at 33°C for 30 min and then maintained at room temperature in oxygenated normal ACSF until needed (Saint Jan & Westbrook, 2007; Fadool et al. 2011).

Neuronal slices were visualized at 10× and 40× using an Axioskop 2FS Plus microscope (Carl Zeiss Microimaging, Inc., Thornwood, NY, USA) equipped with infrared detection capabilities (Dage MT1, CCD100). Electrodes were fabricated from borosilicate glass (Hilgenberg no.1405002, Malsfeld, Germany) to a diameter of approximately 2 μm to yield pipette resistances ranging from 4 to 7 MΩ. Membrane voltage and current properties were generated and acquired using the same amplifier and software as for HEK 293 electrophysiology. The analog signal was filtered at 2 kHz and minimally digitally sampled every 100 μs. Positive pressure was retained while navigating through the olfactory bulb laminae until a high resistance seal (Rc = 1.2–5.1 GΩ) was obtained on a positionally identified mitral cell in the slice (Fadool et al. 2011). The morphology and biophysical properties of the neurons were used to distinguish mitral cells from tufted cells. In addition, thy 1 YFP transgenic mice were a good tool to secondarily confirm cell identity as in Fadool et al. 2011 (see micrograph in Fig. 1). The whole-cell configuration was established by applying gentle suction to the lumen of the pipette while monitoring resistance. Each mitral cell was first sampled for adequate resting potential (less than −55 mV) and proper series resistance (less than 40 MΩ) prior to initiating a series of current-clamp recordings. After determination of the natural resting potential, the membrane potential was then adjusted to −65 mV by injecting a few picoamperes of current so that data acquisition across cells could be statistically evaluated from the same potential. Cells that failed to have a resting membrane potential of at least −50 mV or an input resistance of at least 150 MΩ were discarded from analysis due to biophysical indicators of poor health.

Perithreshold current level was determined by incrementally injecting 1 or 2 s-long, 25 pA steps of current every 10 or 20 s, starting at −50 pA. Following the determination of spike threshold, cells were then stimulated with a long, perithreshold current step (typically ranging from 5 to 75 pA) of 4000 to 5000 ms every 10 to 20 s to acquire spike frequency data, prior to and following glucose concentration changes. Generally recordings were acquired for a minimum of 20 min following a glucose concentration change to allow time for stabilization. Latency to first spike, spike frequency (calculated throughout step depolarization), intraburst frequency (calculated during spike cluster), interspike interval (ISI; calculated within a spike cluster) and action potential cluster length were measured as previously described (Balu et al. 2004; Fadool et al. 2011; Mast & Fadool, 2012). A cluster was defined as three or more consecutive spikes with an ISI of 100 ms or less as established by Balu et al. (2004). Since mitral cell firing is intrinsically intermittent and is characterized by variable spike clusters, classical means of computing spike timing variability, such as peri-stimulus time histograms, were less suitable for the behaviour of these neurons and therefore alternative means of spike analyses were applied as described (Balu et al. 2004).

Data analysis and statistics

All electrophysiological data were analysed using pCLAMP 9 (Axon CNS, Molecular Devices), Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA), and Igor Pro 6.12A (WaveMetrics Inc., Portland, OR, USA) with the NeuroMatic version 2 plug-in (written by Jason Rothman). The pipette capacitance was electrically compensated through the capacitance neutralization circuit of the Axopatch 200B amplifier. Likewise, series resistance compensation was used to electrically reduce the effect of pipette resistance. Voltage-clamp traces were subtracted linearly for leakage conductance. Resting membrane potentials were corrected for a −14 mV junction potential offset.

For voltage-clamp experiments, the peak transient current was defined as the greatest current evoked after voltage activation. The inactivation of the macroscopic current (Iiact) was fitted to the sum of two exponentials (y = γ0 + A1exp(−x/t1) + A2exp(−x/t2)) by minimizing the sums of squares, where y0 was the Y offset, t1 and t2 were the inactivation time constants, x is the time, and A1 and A2 were the amplitudes. The two inactivation time constants were combined by multiplying each by its weight (A) and summing as described previously (Cook & Fadool, 2002; Tucker & Fadool, 2002). The deactivation of the macroscopic current (Iiact) was fitted similarly, but to a single exponential (y = γ0 + A1exp(−x/t1)). To measure changes in the voltage at half-activation (V1/2) and channel conductance (k), the applied voltage was stepped in 5 mV depolarizing increments from −90 mV to −5 mV with a pulse duration of 50 ms and an inter-pulse interval of 10 s. The resulting peak tail currents were plotted against the V1/2, and fitted with a Boltzmann expression. The Boltzmann equation used for fitting was: y = [(A1− A2)/(1 + exp(X − X0)/dx)] + A2, where the...
steepness of the voltage dependence was determined as $d_1$, $A_1$ was the initial $y$ value and $A_2$ was the final $y$ value. $X$ was time and $X_0$ was the centre between the two limiting values $A_1$ and $A_2$ (Cook & Fadool, 2002; Tucker & Fadool, 2002).

For data generated in the HEK cell electrophysiology experiments, statistically different means were determined by a one- or two-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls or Bonferroni post hoc test. When evaluating statistically significant differences across paired data (prior to and following glucose solution change within a cell) generated in the olfactory bulb slice configuration, either a blocked factorial design ANOVA or a paired $t$ test was performed depending upon the number of treatment groups. In experiments in which a family of current steps were used to evaluate changes in frequency, latency to 1st spike, cluster length, and within-cluster spiking frequency, the responses were characterized by fitting the data with either linear or non-linear regressions. Statistical differences in the current–feature relationships before and after changes in glucose concentration were determined for linear regression results by analysis of covariance (ANCOVA) and for non-linear regression results with a sum-of-squares $F$ test. If the current–feature relationship was unable to achieve an $r^2$ value (or the Pearson Coefficient of Determination) of at least 0.4 with at least one type of regression or the fits were of different types, a two-way ANOVA followed by a Bonferroni multiple comparisons post hoc test was used. Statistical significance was determined at the 95% confidence level. All reported values are mean ± standard error of the mean (SEM).

Results

Kv1.3 is sensitive to D- but not L-glucose in a concentration-dependent manner

To determine if Kv1.3 was sensitive to glucose in a metabolically dependent manner, HEK 293 cells expressing Kv1.3 channels were voltage clamped in the presence of varying glucose concentrations. Four glucose concentrations were chosen to mimic hypo-glycaemia (0 mM), normoglycaemia (5 mM), postprandial glycaemia (10 mM), and hyperglycaemia (20 mM) in the periphery. Two isoforms of glucose were chosen to distinguish between metabolic-byproduct-mediated modulation (D-glucose) and direct glucose interactions.

Figure 1. Glucose modulates voltage-activated currents in Kv1.3-expressing HEK 293 cells

A, representative cell-attached voltage-clamp records of Kv1.3 channels heterologously expressed in HEK 293 cells following a 30 min incubation in 0, 5, 10 and 20 mM D-glucose-containing bath solution. Patches were held at –90 mV ($V_h$) and stepped in 20 mV increments from –80 mV to +60 mV. The pulse duration was 1000 ms with an interpulse interval of 60 s. B, bar graph of the mean peak current amplitude for a population of cells recorded as in A, calculated at the –90 to +60 mV step. Number (n) of recordings noted, values represent mean ± SEM in this and subsequent figures. Significantly different means are denoted by different lowercase letters as determined by a one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls post hoc test, $P \leq 0.05$. C, current–voltage relationship plotted for a population of cells as in A for each D-glucose concentration. $n = 4–6$ cells/concentration.
Kv1.3 channels were found to be sensitive to D-glucose in a concentration-dependent manner that followed a bell-shape curve (Fig. 1A and B). The largest voltage-activated peak current amplitude was observed for patches in the 10 mM D-glucose concentration (reported as X-fold change in current over that measured for 0 mM D-glucose; 5 mM, 1.22 ± 0.37 (n = 7); 10 mM, 2.49 ± 0.75 (n = 12); 20 mM, 0.68 ± 0.10 (n = 6); significantly different, one-way ANOVA followed by a Student–Newman–Keuls post hoc test, α ≤ 0.05) (Fig. 1B). D-Glucose modulated the Kv1.3 voltage-activated current but did not change the voltage dependence of Kv1.3 as shown in the current–voltage relationship in Fig. 1C. Neither of the metabolically inactive forms of glucose, L-glucose (10 mM: 1.24 ± 0.11, n = 9) or 2-deoxyglucose (10 mM: 0.92 ± 0.08, n = 6) had a significant effect on Kv1.3 peak current when compared with 0 mM (arc-sine transformation for percentile data, Student’s t test, P < 0.05). When comparing D- and L-glucose isoforms for peak current amplitude in relationship to molarity of glucose, significance was measured at the 10 mM glucose level (two-way ANOVA across

Figure 2. D-Glucose, but not L-glucose, modulates Kv1.3 voltage-activated currents in HEK 293 cells
Voltage-clamp analysis of Kv1.3 channels heterologously expressed in HEK 293 cells following a 30 min incubation in D-glucose (filled bars) or L-glucose (open bars) modified bath solutions. All biophysical properties measured in response to a step depolarization to +60 mV from a V_h of −90 mV as in Fig. 1B. A, bar graph of the voltage-activated peak current normalized to that recorded in 0 mM glucose (within a transfection) vs. glucose concentration. Bar graphs of the kinetics of inactivation (t_{inact}; B), kinetics of deactivation (t_{deact}; C), voltage dependence (k; D), and voltage at half-activation (V_{1/2}; E) with respect to glucose concentration. Bars labelled with different lowercase letters indicate significantly different means (example, a is significantly different from b but not ab) as determined by two-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test, P < 0.05. n = number of recordings.
concentration and treatment groups, Bonferroni post hoc test, \( \alpha \leq 0.05 \), Fig. 2A). No other biophysical property, including voltage dependence \((k)\), kinetics of inactivation \((t_{\text{inact}})\) or deactivation \((t_{\text{deact}})\), or voltage at half-activation \((V_{1/2})\), exhibited any mean significant difference when channels were recorded in D- vs. L-glucose-based bath solutions (one-way ANOVA within glucose isoform and two-way ANOVA comparing isoform and concentration, Fig. 2B–E). These data indicate that for cloned Kv1.3 channels, glucose modulates current amplitude while other biophysical properties involved in channel movement or gating are unchanged. Modulation is concentration dependent, and is restricted to the metabolically active D-glucose isoform.

Mitral cells are glucose sensitive

Kv1.3 channels drive the duration of the action potential spike train, the interspike interval (ISI), latency to the first spike, and the pause duration between spike clusters in mitral cells of the olfactory bulb (Fadool et al. 2011; Mast & Fadool, 2012). Because the typical working concentration of D-glucose in the ASCF used to isolate the olfactory bulb and the external recording bath solutions reported for slice electrophysiological preparations of the olfactory bulb range between 10 and 22–25 mM glucose (Dong et al. 2009; Ma & Lowe, 2010; Borisovska et al. 2011; Lui et al. 2011; Masurkar & Chen, 2011; Giridhar & Urban, 2012; Gire et al. 2012), we initiated our investigation of mitral cell glucose sensitivity using the two extremes of 0 and 22 mM glucose. Regardless of the order of solution presentation, mitral cell firing frequency was found to increase or decrease in response to a change in glucose (change in spike frequency was not significantly different across solution order, 0 to 22 mM, 179.7 ± 34.6% change \((n = 6)\); 22 to 0 mM, 178.7 ± 27.7% \((n = 18)\); Student’s \(t\) test, arc-sine transformation for percentage data, \( \alpha \leq 0.05 \)). Mitral cells were heterogeneous, therefore, in their polarity of response to glucose modulation (inhibition vs. excitation).

A family of evoked action potentials recorded in response to increasing current injection is demonstrated for a glucose-inhibited (Fig. 3) and a glucose-excited cell (Fig. 4), respectively. Time plots of spike frequency for each of these types of mitral cells are shown in panel B of these figures to illustrate the rapid time course (within 30 s) of the modulation, which then stabilized quite rapidly within 60–90 s. Of the 17 cells examined, 76% of the cells were inhibited by high glucose and demonstrated a decrease in spike firing frequency with elevated glucose (Fig. 4C, 0 mM glucose, 25.4 ± 2.4 Hz vs. 22 mM glucose, 12.6 ± 2.16 Hz, 100 pA current injection, paired \( t \) test, \( P = 0.0002 \), \( n = 13 \)). The remaining 24% were excited by high glucose and demonstrated an increase in firing frequency with elevated glucose (Fig. 3C, 0 mM glucose, 3.4 ± 1.7 Hz vs. 22 mM glucose, 19.03 ± 4.4 Hz, 25–100 pA current injection, paired \( t \) test, \( P = 0.022 \), \( n = 4 \)).

Because the onset of glucose modulation appeared to be very rapid, within 15–30 s, we wanted to next determine if we could rapidly reinstate the original mitral cell firing frequency by typical washout. Cells were first stimulated in either 0 or 22 mM glucose and then stepped to the opposite concentration to monitor firing frequency prior to attempting washout in the original glucose concentration. An example of the firing activity of one of 13 cells in which a 10–20 min washout was attempted is demonstrated in Fig. 5A in the form of a raster plot with accompanying frequency line plot. Representative traces taken under control (22 mM), solution switch (0 mM), and washout (22 mM) intervals are shown in Fig. 5B, in which a noted decrease in action potential cluster length and number of spikes per cluster in response to a decrease in glucose (22 mM to 0 mM) was reversed following a washout with 22 mM glucose. Original action potential firing frequency was re-established in 8 of the 13 cells following the washout as shown as a graph in Fig. 5C and quantified in Fig. 5D (Friedman test, Dunn’s multiple comparison follow-up test, \( \alpha \leq 0.05 \)). There was a trend observed in these experiments for mitral cells with higher basal firing frequencies to increase firing frequency when switched to low glucose and those with lower basal firing frequency to decrease firing frequency under similar conditions (Fig. 5C).

Neuromodulation of glucose-inhibited mitral cells

Because the majority of mitral cells were inhibited by an increase in glucose concentration, we elected to further study the potential mechanism of this neuromodulation and did not further explore the glucose excitatory subset of mitral cells. An analysis of action potential shape did not demonstrate any significant differences in spike amplitude or width at half-maximum amplitude for cells recorded in 22 mM vs. 0 mM glucose (Fig. 6A–C). Spike timing, however, was significantly modified. Mitral cells recorded in 22 mM glucose exhibited significantly lower spike firing frequencies across a family of injected current steps and had an increased latency to the first action potential with a 50 and 75 pA current injection compared to those measured in the presence of 0 mM glucose (Fig. 6D: significant difference was determined by linear regression followed by an ANCOVA to determine a difference in elevation, \( P < 0.0001 \); Fig. 6E: significant difference (*) was determined by two-way repeated measures ANOVA with a Bonferroni post hoc test, \( P < 0.05 \)). These changes in spike timing were attributed to a significant decrease in number of spikes per action potential cluster and a concomitant decrease in cluster length (Fig. 6F: significant difference was determined by linear regression followed by an ANCOVA to determine a difference in
Physiological concentrations of glucose affect mitral cell spiking

Extracellular glucose concentrations in the brain fluctuate between 0.2 mM and 5 mM during systemic hypo- and hyperglycaemia (Silver & Erecinska, 1994). To assess whether physiologically relevant levels of glucose directly cause changes in mitral cell firing, we elected to compare spike firing properties in 2.5 mM vs. 22 mM bath solutions and in 0.1 mM vs. 2.5 mM bath solution conditions. The first condition was designed to mimic a mid-range extracellular brain glucose concentration in comparison to a diabetic state. The second condition was designed to mimic a hypoglycaemic state in comparison to a post-prandial, fed state. In both of these experimental conditions, we observed a decrease in spike firing frequency in response to increased glucose (Fig. 7C–F).

In the first experimental condition (2.5 mM vs. 22 mM), 6 of 7 neurons were significantly inhibited by glucose, and in the second experimental condition (0.1 mM vs. 2.5 mM), 5 of 7 neurons were significantly inhibited by glucose (paired t test, \( \alpha \leq 0.05 \), respectively). The remaining neurons did not exhibit a firing frequency change in response to a change in glucose concentration.

Because the cloned Kv1.3 channel exhibited the largest current magnitude at 10 mM in the voltage-clamped configuration (Figs 1A and 2A), we predicted that a 10 mM glucose bath solution would concomitantly have the least change in action potential excitability attributed to this concentration of glucose. We therefore compared a third experimental condition (10 mM vs. 22 mM) to the two physiologically relevant conditions in Fig. 7 (above), and as anticipated, a change to a 10 mM bath solution failed to significantly inhibit firing frequency (paired t test, \( \alpha \leq 0.05 \), respectively).
to significantly alter firing frequency compared to that in 22 mM glucose (Fig. 7A and B).

**Responses of mitral cells to glucose in the presence of synaptic blockers**

Previous studies have demonstrated that subtypes of glucosensing neurons in the ventral medial nucleus of the hypothalamus do not have intrinsic glucosensing properties. Rather, their action potential frequencies and membrane properties are altered by presynaptic inputs in response to changes in extracellular glucose (Song *et al.* 2001). We have previously demonstrated that modulation of mitral cell firing by insulin or brain-derived neurotrophic factor is intrinsic and is not affected by the inclusion of synaptic blockers (Fadool *et al.* 2011; Mast & Fadool, 2012). We therefore wanted to test whether glucosensing properties of mitral cells were intrinsic or involved synaptic transmission. To eliminate synaptic inputs, we included a cocktail of synaptic blockers in our bath recording solutions upon breakthrough to the whole-cell configuration (glutamatergic receptor blockers NBQX and D-APV, and the GABAergic receptor blocker gabazine). In comparing spiking timing of cells in 0 mM vs. 22 mM glucose recording conditions, we observed a complete blockade of glucose-induced inhibition of mitral cell firing frequency (Fig. 8A). Unlike that measured in the absence of synaptic blockers, change to higher glucose concentrations no longer significantly modulated spike firing frequency, latency to the first action potential, within-cluster spiking frequency, or action potential cluster length (Fig. 8B–E). Repeating this same experimental protocol, but including the synaptic blockers individually as opposed to a cocktail, demonstrated that D-APV or gabazine alone effectively blocked glucose-induced inhibition of mitral cell firing (Fig. 9A and B–E); however, NBQX was ineffective in blocking glucose modulation (Fig. 9A and F–G). Significant differences in spike frequency data in Fig. 9F were determined by comparison of linear regression

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**Figure 4. High glucose concentration decreases action potential firing frequency in 13 of 17 mitral cells**

*A.** representative evoked action potential activity in a mitral cell that was inhibited by D-glucose. Same recording configuration and notations as Fig. 3. *B.* plot of the spike frequency (Hz) over time (min) for a representative mitral cell injected with a perithreshold current of 100 pA held for 4000 ms and applied every 20 s for a 10 min recording interval. *C.* line plot of spike frequency changes in response to a change in glucose concentration for 13 mitral cells found to be inhibited by high glucose. *Significantly different means as determined by two-tailed, paired t test, \( P = 0.0002, n = 13. \) Symbols as in A.
with ANCOVA, $P = 0.0003$ for elevation. Significant differences (*) in cluster length data in Fig. 9G were determined by repeated measures two-way ANOVA followed by a Bonferroni multiple comparisons post hoc test. Taken together, these results indicate that synaptic inputs contributed by GABA\_A and NMDA receptors, rather than AMPA and kainite receptors, are a necessary component of glucose sensing in mitral cells that are inhibited by glucose.

**Mitral cells fail to be modulated by glucose in Kv1.3\(^{-/-}\) mice**

Given our initial discovery that extracellular d-glucose modulates Kv1.3 voltage-activated currents in a heterologous expression system, we wanted to test for glucosensing in mitral cells under pharmacological block or absence of the channel. Performing analogous experiments to those described in Fig. 6, spike firing frequency was measured in mitral cells in which slices had been preincubated for 15 min in 1 nM margatoxin (MgTx). This scorpion-derived toxin is a small peptide known to have high affinity binding to the vestibule of the Kv1.3 channel that is selective over that of other Kv family members (Knaus et al. 1995). We have previously demonstrated its effectiveness and selectivity for Kv1.3 block in both olfactory bulb cultures and slice preparations (Fadool & Levitan, 1998; Colley et al. 2004; Mast & Fadool, 2012). Of the six cells recorded, none were sensitive to a 22 vs. 0 mM glucose concentration change (fold change in firing frequency $0.97 \pm 0.16$, $n = 6$, 50 pA current injection, paired $t$ test, $\alpha \leq 0.05$; Fig. 10A–E). Moreover, we compared these results testing for glucose modulation during a pharmacological block of Kv1.3 to that acquired in olfactory bulb slices prepared from Kv1.3\(^{-/-}\) mice. Of the 15 cells recorded in Kv1.3\(^{-/-}\) mice, none were sensitive to a 22 mM vs. 0 mM glucose concentration change (fold change in firing frequency $1.02 \pm 0.16$, $n = 15$, 50 pA current injection, paired $t$ test, $\alpha \leq 0.05$; Fig. 10F). There were no significant differences in firing frequency, latency to first action potential, number of spikes per cluster, or cluster length in Kv1.3\(^{-/-}\) mitral cells recorded in 0 vs. 22 mM glucose (Fig. 10F–J). These data suggest that the Kv1.3 channel is a necessary component of the

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**Figure 5. Modulation by glucose is rapid and reversible**

A, raster plot (left) and associated spike frequency plot over time for the evoked action potential activity in a mitral cell that was held in 22 mM glucose, then switched to 0 mM glucose, and then reversed back to 22 mM glucose as a washout. B, representative action potentials acquired at each of the three time points designated as a, b and c in the recording shown in A. C, line plot of spike frequency changes in response to sequential glucose changes as in A for 8 of 13 mitral cells that returned to original firing frequency as observed in the starting solution. D, histogram plot of the mean spike frequency change as a function of the percentage of the original solution (Friedman test, Dunn’s multiple comparison follow-up test, $\alpha \leq 0.05$).
Figure 6. Mitral cell spike frequency, first latency and train duration, but not action potential shape, are affected by glucose.

Action potential shape and spike frequency analysis for a population of mitral cells that were inhibited by glucose. A, representative action potentials generated from a mitral cell that was recorded using the same protocol as in Fig. 4B. Line graph of the mean action potential amplitude (Spike amplitude; B), width at half-maximum amplitude (Width at 1/2 max, C), spike frequency (Frequency, D), latency to first action potential (Latency 1st spike, E), action potentials per duration of the spike train (Intraburst frequency, F) and duration of spike train (Cluster length, G) versus current step as computed for a population of glucose-inhibited mitral cells using the recording configuration and notations as in Fig. 4A. Data in D and F were fitted by linear regression (lines), which were determined to be significantly different by an analysis of covariance (ANCOVA) with respect to elevation but not slope. For data in E, * indicates significantly different means at the 95% confidence interval determined by repeated measures two-way ANOVA followed by Bonferroni post hoc test. For data in G, significant differences in cluster length were determined by comparing one-phase association least squares fits with a sum-of squares F test, $P = 0.0056$. $n = 12$ recordings/plot.
Discussion

Kv1.3 channels represent a central scaffold upon which many tyrosine kinases and associated signalling proteins converge to modulate channel and neuronal activity. We now demonstrate that Kv1.3 channels and Kv1.3-expressing neurons of the olfactory bulb can be modulated by a molecule not directly linked to phosphorylation, but one central to metabolism, namely glucose. Similar to other glucosensing neurons in other brain regions, glucose modulation of mitral cells is rapid and, like reported glucosensors in the hypothalamus and hippocampus, it is quite readily reversible. Modulation appears to span a large dynamic range, from hyperglycaemic levels reaching near diabetic ketosis states (22 mM) to that of hypoglycaemic, pre-prandial levels (0.1 mM) and requires both synaptic transmission and the Kv1.3 channel. Such a neuromodulatory mechanism would allow the olfactory system to regulate its olfactory code in terms of spike generation in the olfactory bulb, based upon energy availability.

Our studies of cloned Kv1.3 heterologously expressed in HEK 293 cells provide evidence that the channel is sensitive to D-glucose in a concentration-dependent manner and that it is modulated by the metabolically active form of the glucose isof orm. Kv1.3 channels have previously been shown to be modulated by ATP (Chung & Schlichter, 1997a) and reactive oxygen species (ROS) (Duprat et al. 1995; Cayabyab et al. 2000), both of which are byproducts of glucose metabolism. ATP is the energy currency for cellular processes and under hypoglycaemic conditions, ATP availability is low. ROS, such as superoxides, are byproducts of oxidative phosphorylation and during normal metabolism, only about 0.1% of total oxygen used as a proton acceptor during metabolism becomes an ROS (Rolo & Palmeira, 2006). ROS production is high under hyperglycaemic conditions due to changes in electron transport coupling and is thought to be a major cause of hyperglycaemia-induced diabetic complications (Rolo & Palmeira, 2006). In our HEK 293 cell preparation, Kv1.3 currents were acutely glucose sensitive and currents were highest under conditions mimicking the fed state (10 mM) and lowest at the upper and lower extremes where one would anticipate changes in ATP/ROS production. Albeit a parallel drawn from non-neuronal cells, our result could be in keeping with an indirect mechanism found in T lymphocytes whereby Kv1.3 currents have been previously demonstrated to be sensitive to ATP (Chung & Schlichter, 1997a) and ROS (Duprat et al. 1995; Cayabyab et al. 2000).

Figure 7. Mitral cells respond to changes in glucose within estimated physiological ranges

Representative evoked action potential activity in three different mitral cells that were inhibited by glucose when comparing 10 mM vs. 22 mM glucose conditions (A), 2.5 mM vs. 22 mM glucose conditions (C), or 0.1 mM vs. 2.5 mM glucose conditions (E). A, C and E, paired recordings were made by current clamping a mitral cell in the whole-cell configuration whereby current injections were stepped in 25 pA intervals for 1000 ms every 10 s to evoke a train of action potentials during exposure to a bath solution containing low (filled circles) and then high glucose (open circles) or vice versa. A cell was recorded for approximately 10–20 min in a given bath solution prior to transitioning to a new solution. B, D and F, graph of the spike frequency versus glucose concentration for the three experimental conditions, respectively, taken at the 125 pA current step. Each line is an individual paired recording where spike frequency determinations were made at the 10 min time point following a solution change. *Significantly different by paired t test, P ≤ 0.05.
Figure 8. Glucose-induced decrease in mitral cell firing is blocked in the presence of synaptic blockers

A, representative evoked action potential activity in a mitral cell that was inhibited by glucose when comparing 0 mM vs. 22 mM glucose conditions in the presence of a mixture of synaptic inhibitors containing NBQX, d-APV and gabazine. The paired recording was made by current clamping the cell in the whole-cell configuration whereby current injections were stepped in 25 pA intervals for 1000 ms every 10 s to evoke a train of action potentials during exposure to a bath solution containing 0 mM (filled circles) and then 22 mM (open circles) or vice versa. A cell was recorded for approximately 10–20 min in a given bath solution prior to transitioning to a new solution. B–E, spike frequency analysis, notation, and statistics as in Fig. 6 for a population of glucose-inhibited mitral cells recorded in the presence of the synaptic blockers. No statistically significant differences were found. n = 8 recordings.
Figure 9. Glucose-induced decrease in mitral cell firing is blocked in the presence of gabazine and D-APV but not NBQX

A, representative evoked action potential activity in three different mitral cells that were inhibited by glucose when comparing 0 mM vs. 22 mM glucose conditions in the presence of synaptic inhibitors applied individually as specified. Same recording protocol and notation as in Fig. 8. Spike frequency and cluster length analysis, notation and statistics as in Fig. 6 for a population of glucose-inhibited mitral cells recorded in the presence of the GABA<sub>A</sub> receptor blocker gabazine (n = 6; B and C), NMDA and kainite receptor blocker D-APV (n = 5; D and E), or the AMPA and kainite receptor blocker NBQX (n = 4; F and G). Linear regression lines of data in B, D and F were compared using an ANCOVA for differences in slope or elevation (B, gabazine treated P > 0.05; D, D-APV treated P > 0.05; F, NBQX treated, P = 0.0003). Data in C, E and G were compared using a repeated measures two-way ANOVA followed by a Bonferroni multiple comparisons post hoc test (C, gabazine treated not significantly different; E, D-APV treated, not significantly different; G, NBQX treated, current × treatment P > 0.05, current P = 0.012, treatment, P = 0.0002).
Figure 10. Mitral cell modulation by glucose is absent following pharmacological block or in Kv1.3−/− mice

A, representative evoked action potential activity in a mitral cell in which 1 mM margatoxin (MgTx) was applied to the bath 20 min prior to testing for glucose modulation. The cell was current clamped in the whole-cell configuration whereby current injections were stepped in 25 pA intervals for 1000 ms every 10 s to evoke a train of action potentials during exposure to a bath solution containing 0 mM glucose (filled circles) and then 22 mM glucose (open circles). Quantification of spike frequency (B), intraburst frequency (C), latency to 1st spike (D), and cluster length (E) for a population of 6 mitral cells treated as above. Linear regression lines for 0 and 22 mM glucose-treated cells in B, D and E were analysed with an ANCOVA. Data in C were analysed with a repeated measures two-way ANOVA. No statistical differences were found. F–J, same recording conditions and analyses as in A–E, but displaying representative evoked action potential activity in a mitral cell acquired from a Kv1.3−/− rather than a WT mouse. n = 15 recordings. No statistical differences were found.
It is also possible for glucose to modulate ion channel activity by direct channel glucose interaction (such as direct pore blockade, channel binding or unknown receptor activation and subsequent activation of a cellular signalling cascade). Glucose could indeed interact with Kv1.3 directly in the HEK 293 cells, but in mitral cells there are other known glucose/ATP/ROS-sensitive channels such as Kir6.2 (Zhou et al. 2002), KCa1.1 (Sausbier et al. 2006) and Na\(^+\)-activated K\(^+\) channels (Slick/Slack) (Lu et al. 2010). For example, the large conductance Ca\(^{2+}\)-activated K\(^+\) channel KCa1.1 has been found to be inhibited by hyperglycaemic-induced increases in ROS when expressed in HEK 293 cells as well as in human coronary smooth muscle cells (Lu et al. 2006). The inward rectifier, Kir6.2 or K\(_{ATP}\) channel, is ATP sensitive and the ether-à-go-go-related gene (HERG) K\(^+\) channel or Kv11.1 has been shown to be suppressed at both hypoglycaemic (0 mM) and hyperglycaemic (10–20 mM) glucose concentrations, with the highest current at normoglycaemic (5 mM) concentrations (Zhang et al. 2003). The Na\(^+\)-activated K\(^+\) channel, Slack, is activated by high levels of sodium and low levels of ATP typically found in hypoglycaemic conditions (Bhattacharjee & Kaczmarek, 2005). Because mitral cells from Kv1.3\(^{-/-}\) mice are insensitive to glucose, one might surmise that these other metabolic-sensitive potassium channels are not involved in (downstream) glucose sensing; however, there are many changes in protein expression in the Kv1.3\(^{-/-}\) mice (Fadool et al. 2004; Lu et al. 2010), including elevation of Slack channels, that would confound this interpretation. It is unclear, therefore, if Kv1.3\(^{-/-}\) are completely insensitive to glucose due to loss of channel conduction, or if glucose sensitivity may be masked by multiple changes in the expression of ion channels and associated protein partners. The pharmacological elimination of the Kv1.3 component in mitral cells due to MgTx incubation of wild-type slices, however, was able to render the neurons insensitive to glucose, suggesting that Kv1.3 is indeed an important part of the glucose detection mechanism in these neurons. Using voltage protocols and favourable ionic compositions to study other potassium channels would allow the examination of other potential mechanisms for glucose sensing. Future electrophysiological experiments, therefore, could be designed incorporating specific Kir6.2, KCa1.1 and Slack channel blockers to tease apart the contribution of other potential glucose/metabolic-sensitive targets in these neurons.

In order for glucosensing to be physiologically relevant, brain neurons must be able to respond across a dynamic range as well as have refined sensitivity to concentration. According to the American Diabetes Association (2012; http://www.diabetes.org/), the inter-meal blood glucose levels of healthy individuals is between 3.8 and 7.2 mM, and rises to around 10 mM within 30 min after feeding. This increase in blood glucose stimulates insulin release that in turn stimulates glucose uptake into cells. Glucose is thereby removed from the blood stream, and blood glucose levels return to preprandial levels. Diabetic patients have higher inter-meal glucose levels that rise to between 10 and 20 mM just after a meal and are much slower to return to baseline without insulin treatment. Hypoglycaemic conditions are considered to be below 3.8 mM.

On average, brain extracellular glucose concentrations follow blood glucose levels at approximately 30% of those found in the blood (Silver & Erecinska, 1994; Seaquist et al. 2001; McNay & Gold, 2002) but vary from region to region and with neuronal activity. For example McNay and Gold, in a series of zero flux microdialysis experiments, found that the resting extracellular glucose level in the rat hippocampus was 1.2 mM while that in the striatum was 0.7 mM (McNay & Gold, 1999; McNay et al. 2001). During memory tasks, the hippocampal extracellular glucose levels decrease in a concentration-dependent manner with increasing difficulty of the task (McNay et al. 2000), whereas there was no change in striatum levels (McNay et al. 2000). These results suggest that extracellular brain glucose levels are compartmentalized, possibly due to differences in vascularization, glucose transporter expression, or acute metabolic demand due to neuronal activity.

A more recent study compared the glucose concentration in five discrete brain regions, including the olfactory bulb, with plasma glucose concentrations in anaesthetized rats (Poitry-Yamate et al. 2009). Brain glucose concentrations increased linearly with plasma glucose concentration with a slope of 1.2–0.8 in all five regions with no significant differences among the five regions. In the olfactory bulb, when plasma glucose concentrations were 5, 10 and 20 mM, the olfactory bulb tissue concentrations were calculated to be 1, 2 and 5 \(\mu\)mol g\(^{-1}\), respectively (Poitry-Yamate et al. 2009). Taken together, these results suggest that brain glucose concentrations fluctuate with peripheral glucose concentrations albeit at significantly lower levels (1/3–1/5 brain/plasma; Silver & Erecinska, 1994; Poitry-Yamate et al. 2009). The lower glucose concentrations (0.1–2.5 mM) used in the later part of this study would therefore be on a par with what the olfactory bulb might be naturally encountering. According to Lecoq and colleagues (Lecoq et al. 2009), the olfactory bulb has among the highest capillary densities in the brain due to the energy demands of odourant detection. Odour-induced mitral/tufted cell activity significantly increases metabolic oxygen consumption (Lecoq et al. 2009) and thereby requires a strong demand for glucose and related metabolic substrates. It is also interesting to note that the artificial cerebral spinal fluid used for bath solution in most olfactory bulb slice electrophysiology experiments contains anywhere from 10 to 25 mM D-glucose (Dong

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et al. 2009; Ma & Lowe, 2010; Lui et al. 2011; Borisovska et al. 2011; Masurkar & Chen, 2011; Giridhar & Urban, 2012; Gire et al. 2012). These are much higher concentrations than would ever be seen by the brain in vivo and may even act to suppress evoked action potential discharge as has been seen in a large proportion of mitral cells in our study.

Although our data demonstrate an involvement in synaptic transmission and Kv1.3 in the capacity for mitral cells to sense glucose, the precise molecular mechanism is far from clear. The fact that mitral cells fail to show glucose sensitivity in the presence of gabazine or D-APV could suggest a mechanism by which hypoglycaemia normally enhances mitral cell firing as a counter-regulatory response that is mediated through the activation of the mitral-granule cell dendrodendritic synaptic connections to reduce inhibitory GABAergic tone. Another possibility is a mechanism regulated by glucose transporters. According to the Allen Brain Atlas, the sodium-dependent glucose co-transporters SGLT1 and SGLT4 are expressed in a subset of mitral cells (Allen Institute for Brain Science, 2010). These sodium glucose co-transporters bring in 1–2 sodium ions per glucose molecule and could therefore depolarize a mitral cell in the same way they do in a subset of glucose-sensing neurons of the hypothalamus (Gonzalez et al. 2008, 2009). The glucose transporters GLUT1, 3, 4, 6, 8 and 12 are also expressed in mitral cells resulting in more potential points of glucose entry (Allen Institute for Brain Science, 2010; Palouzier-Paulignan et al. 2012). Interestingly, genetic deletion or block of the Kv1.3 channel has been shown to increase GLUT4 translocation to the membrane in peripheral tissues (Xu et al. 2004; Li et al. 2006, 2007) and it is unknown if a similar mechanism might exist in neurons.

Regardless of the precise molecular mechanism of glucosensing in the olfactory bulb, it remains that mitral cells are modulated by changes in glucose concentration, and as such, represent a conduit for sensing energy availability in this brain region. Because olfactory bulb glucose concentrations fluctuate with peripheral concentrations, albeit at much lower concentrations, detection of odour information can be state dependent by modulating firing frequency and the olfactory code. Whether this state changes based upon food availability or metabolic disease (diabetes/obesity), mitral cells would be expected to modulate their sensitivity based upon changes in total energy availability. How precisely Kv1.3 senses energy molecules is an important and intriguing role for an ion channel protein that requires further experimental attention.

References


Mitrat cell and Kv1.3 glucose sensitivity


Author contributions

All experiments were performed in the laboratory of D.A.F. at Florida State University. K.T. and D.A.F. were responsible for the conception and design of the experiments. K.T., S.C., N.T. and M.H. were responsible for the collection of the data. All authors were responsible for the analysis and interpretation of the data. K.T., S.C. and D.A.F. drafted the article and revised it critically for important intellectual content. All authors approved the final version of the manuscript.

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