View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Open Research Exeter

Diabetologia (2012) 55:2432–2444
DOI 10.1007/s00125-012-2617-y

ARTICLE

Mouse hypothalamic GT1-7 cells demonstrate AMPK-dependent intrinsic glucose-sensing behaviour

C. Beall · D. L. Hamilton · J. Gallagher · L. Logie · K. Wright · M. P. Soutar · S. Dadak · F. B. Ashford · E. Haythorne · Q. Du · A. Jovanović · R. J. McCrimmon · M. L. J. Ashford

Received: 16 January 2012 / Accepted: 21 May 2012 / Published online: 4 July 2012
© The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract
Aims/hypothesis Hypothalamic glucose-excited (GE) neurons contribute to whole-body glucose homeostasis and participate in the detection of hypoglycaemia. This system appears defective in type 1 diabetes, in which hypoglycaemia commonly occurs. Unfortunately, it is at present unclear which molecular components required for glucose sensing are produced in individual neurons and how these are functionally linked. We used the GT1-7 mouse hypothalamic cell line to address these issues.
Methods Electrophysiological recordings, coupled with measurements of gene expression and protein levels and activity, were made from unmodified GT1-7 cells and cells in which AMP-activated protein kinase (AMPK) catalytic subunit gene expression and activity were reduced.
Results Hypothalamic GT1-7 neurons express the genes encoding glucokinase and ATP-sensitive K^+ channel (K_{ATP}) subunits $K_{ir}6.2$ and *Sur1* and exhibit GE-type glucose-sensing behaviour. Lowered extracellular glucose concentration hyperpolarised the cells in a concentration-dependent manner, an outcome that was reversed by tolbutamide. Inhibition of glucose uptake or metabolism hyperpolarised cells, showing that energy metabolism is required to maintain their resting membrane potential. Short hairpin (sh)RNA directed to *Ampkα2* (also known as *Prkaa2*) reduced GT1-7 cell AMPKα2, but not AMPKα1, activity and lowered the threshold for hypoglycaemia-induced hyperpolarisation. sh*Ampkα1* (also known as *Prkaa1*) had no effect on glucose-sensing or AMPKα2 activity. Decreased uncoupling protein 2 (*Ucp2*) mRNA was detected in AMPKα2-reduced cells, suggesting that AMPKα2 regulates UCP2 levels.
Conclusions/interpretation We have demonstrated that GT1-7 cells closely mimic GE neuron glucose-sensing behaviour, and reducing AMPKα2 blunts their responsiveness to hypoglycaemic challenge, possibly by altering UCP2 activity. These results show that suppression of AMPKα2 activity inhibits normal glucose-sensing behaviour and may contribute to defective detection of hypoglycaemia.

Keywords AMPK · Glucokinase · Glucose sensing · GT1-7 cells · Hypoglycaemia · Hypothalamus · K_{ATP} · UCP2

Abbreviations

ACC	Acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
GCK	Glucokinase
GE	Glucose excited
GI	Glucose inhibited
GnRH	Gonadotrophin-releasing hormone
K_{ATP}	ATP-sensitive K^+ channel
K_{ir}	Inwardly rectifying K^+ channel
POMC	Proopiomelanocortin
shCont	Control shRNA
shRNA	Short-hairpin RNA
SUR1	Sulfonylurea receptor 1
UCP2	Uncoupling protein 2

Electronic supplementary material The online version of this article (doi:10.1007/s00125-012-2617-y) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

C. Beall · D. L. Hamilton · J. Gallagher · L. Logie · K. Wright · M. P. Soutar · S. Dadak · F. B. Ashford · E. Haythorne · Q. Du · A. Jovanović · R. J. McCrimmon · M. L. J. Ashford (✉)
Medical Research Institute, Division of Cardiovascular & Diabetes Medicine, Ninewells Hospital & Medical School,
University of Dundee,
Dundee DD1 9SY, UK
e-mail: m.l.j.ashford@dundee.ac.uk

Introduction

The ability of the hypothalamus to sense change in glucose levels is important in the control of feeding, energy expenditure and peripheral glucose homeostasis in mammals. Indeed, for patients with type 1 diabetes, the impairment of central detection of reduced glucose levels (hypoglycaemic unawareness) is a major concern, as this results in a defective counter-regulatory response, leading to severe risk of profound hypoglycaemia and consequent morbidities [1]. Although brain glucose concentrations parallel those of plasma, they are generally much lower. Thus, during euglycaemia, brain glucose concentrations are ~1.0–2.5 mmol/l and, during extreme hyperglycaemia or hypoglycaemia, may reach 5 and 0.2 mmol/l, respectively [2]. To detect changes in brain glucose levels and produce proportionate physiological responses, a neuronal glucose-sensing system is required. Brain regions intimately associated with this role are the hypothalamus, amygdala, basal ganglia and hindbrain, where specific neuron subtypes that respond electrically to acute variations in glucose are situated [3]. The two major subtypes are glucose-excited (GE) and glucose-inhibited (GI) neurons, whereby a hypoglycaemic stimulus results in hyperpolarisation and inhibition and depolarisation and excitation, respectively [3, 4].

The identity of the molecular constituents that confer glucose-sensing properties on these neurons is unclear. This is due to the difficulties associated with intact brain tissue, absence of a transgenic mouse model allowing easy location of glucose-sensing neurons, uncertainties regarding the role of astrocytes, and lack of a suitable cell culture model. Although GE neurons exhibit similar glucose-sensing behaviour to pancreatic beta cells, the glucose concentration range over which electrical responses occur deviates significantly. The responsiveness of beta cells to altered plasma glucose is dependent on the presence of: GLUT2 in rodents (GLUT1 in humans), the high-capacity glucose transporter; GCK, the low-affinity hexokinase isoform, glucokinase; and K_{ATP}, the ATP-sensitive K^+ channel, consisting of the K^+ channel subunit, $K_{ir}6.2$, and the sulfonylurea receptor, SUR1 [5]. All four proteins are produced in hypothalamic cells, but not always in a coincident manner and in conjunction with unequivocal identification of glucose-sensing properties [6, 7]. Thus there is no clear consensus about the molecular definition of glucose sensing in GE neurons. Our knowledge of the molecular constituents that underlie GI neuron glucose-sensing behaviour is even less well advanced [8]. In addition, glucose sensing may not be an intrinsic feature of hypothalamic neuron populations, but may require metabolic support from glial cells, particularly astrocytes [1, 6].

Recent studies have shown that AMP-activated protein kinase (AMPK) is an essential component for detection of hypoglycaemia by pancreatic beta cells and hypothalamic neurons. Thus ablation of the AMPKα2 catalytic subunit from beta cells [9] and subpopulations of GE hypothalamic neurons [10] results in failure of these cells to respond electrically to reduced levels of glucose. Importantly, hypothalamic AMPK plays a key role in the integrative response to central hypoglycaemia detection, with AMPK downregulation suppressing [11], and activation amplifying [12], counter-regulatory responses, respectively.

We show that mouse hypothalamic GT1-7 cells [13] exhibit hypoglycaemia-detecting behaviour typical of GE neurons and utilise a similar array of molecular components to beta cells to elicit an electrical response. Furthermore, like native hypothalamic neuron and beta cell glucose sensors, GT1-7 cells exhibit dependence on AMPKα2 activity for the transduction of a hypoglycaemic signal to an electrical response.

Methods

Cell culture GT1-7 cells (Pamela Mellon, San Diego, California, USA [13]) were maintained in DMEM (Sigma-Aldrich, Gillingham, UK) with 10% FBS (PAA Laboratories, Yeovil, UK) as previously described [14].

Immunoblotting GT1-7 cells, in six-well dishes, were serum-starved for 3 h, and DMEM (low or high glucose) was replaced with normal saline (below) before challenge with glucose. Protein isolation and immunoblotting procedures were as described previously [14]. Briefly, protein lysates were subjected to SDS-PAGE and electrotransferred to nitrocellulose membrane, and immunoreactive proteins were identified by chemiluminescence. Primary antibodies used were: phospho-AMPK (Thr172; 1:1000 dilution) and phosphorylated acetyl-CoA carboxylase (p-ACC; Ser79; 1:1000 dilution) from New England Biolabs, Hitchin, UK; AMPKα2 and AMPKα1 from D.G. Hardie, University of Dundee, Dundee, UK; GK from M. Magnusson, Vanderbilt University, Nashville, Tennessee, USA; $K_{ir}6.2$ (p-Ser385) from L.M. Chuang, Taipei, Taiwan; actin (1:5000 dilution) from Sigma-Aldrich. Gel protein bands were quantified by densitometry, where total density was determined with respect to a constant area, the background was subtracted, and the average relative band density was calculated.

Assay of AMPK activity GT1-7 cells were maintained in 2.5 mmol/l glucose/DMEM and serum and washed in normal saline (2.5 mmol/l glucose), before challenge with 2.5 or 0.5 mmol/l glucose for various times. Cells were lysed in lysis buffer (in mmol/l: 50 Tris-HCl, pH 7.5, 150 NaCl, 50 NaF, 5 sodium pyrophosphate, 1 EDTA, 1 EGTA, 1 dithiothreitol,

0.1 benzamidine) with 0.1 mmol/l phenylmethanesulfonyl fluoride, 5 μg/ml soya bean trypsin inhibitor and 1% (vol./vol.) Triton X-100, and the protein content was determined (BCA assay; Fisher Scientific, Loughborough, UK). AMPK activity was determined as previously described [15] by calculating the difference in counts between AMARA (AMPK substrate: AMARAASAAALARRR)-containing and AMARA-negative samples as nmol ATP incorporated per min per mg peptide. Data were normalised to the control and are expressed as the mean of four to six independent experiments each with three replicates.

AMPK knockdown Lentiviral transduction of cells using non-targeting short hairpin RNA (shRNA; control; SHC202; Sigma-Aldrich) and shRNA targeting *Ampkα1* (also known as *Prkaa1*) and *Ampkα2* (also known as *Prkaa2*) was performed as per the manufacturer's instructions. Briefly, GT1-7 cells were grown in poly-L-lysine-coated 12-well dishes to ~50% confluence. Hexadimethrine bromide (10 μg/ml) and 40 μl lentiviral particles were added to each well, and, after 24 h, the mixture was replaced with fresh medium. Cells were grown to ~80% confluence and selected using puromycin hydrochloride (5 μg/ml). All data presented are from three to four independent cell lines, each generated in parallel to a control, and comparisons are between the knockdown line of interest and their control. Knockdown of AMPKα1 and AMPKα2 was screened by western blot and assay of radiolabelled kinase activity. A panel of five clones targeting AMPKα1 and AMPKα2 was used for screening, with clones XM_139298.4-1396s1c1 (AMPKα1) and XM_131633.3-858s1c1 (AMPKα2) providing the best knockdown.

Gene expression studies mRNA was quantified using real-time quantitative RT-PCR as described previously [9, 16]. mRNA was extracted from GT1-7 cells or mouse brain, heart or liver using Tri reagent (Sigma-Aldrich) according to the manufacturer's protocol. cDNA was prepared using 1 μg RNA reverse transcribed with Superscript II kit (Life Technologies, Paisley, UK) or ImProm-II reverse transcriptase (Promega, Madison, WI, USA) with oligo(dT) priming and RNase treatment. mRNA expression was analysed using an ABI Prism 7500 or ABI 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA) or an iCycler iQ TM Multicolor Real-Time system (Bio-Rad, Hercules, CA, USA) using primer/probe sets designed (Applied Biosystems, Paisley, UK) to target *Slc2a1*, *Slc2a2*, *Slc2a3*, *Slc2a4* (solute carrier family 2 [facilitated glucose transporters 1–4]), *Hk1*, *Hk2*, *Hk3* and *Gck*, *Slc16a7* (neuronal monocarboxylate transporter), *Abcc8*, *Abcc9* (sulfonylurea receptors 1 and 2), and *Kcnj8* and *Kcnj11* ($K_{ir}6.1$ and $K_{ir}6.2$), and data were analysed by the $2^{-\Delta C_t}$ method [16]. Levels of *Ucp2* mRNA under control and AMPKα2 knockdown are expressed relative to 18S RNA. For detection of *Gck* mRNA, tissues were homogenised in Trizol reagent, and 1 μg RNA reverse transcribed as above. PCR was carried out with first-strand cDNA with primers for mouse pancreas-type GCK (forward, TGGAGGCCACCAAGAAGGAAAAG; reverse, GCATCTCGGAGAAGTCCCACGATG).

Electrophysiology GT1-7 cells were superfused at room temperature (22–25°C) with saline (in mmol/l): 135 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 or 2.5 glucose (pH 7.4). Membrane potentials were recorded using perforated-patch or whole-cell current-clamp configurations, and currents by whole-cell voltage clamp. In whole-cell experiments, cells were maintained in current-clamp mode to monitor resting membrane potential, with short excursions into voltage clamp to obtain current–voltage relations. Current- and voltage-clamp data were collected and analysed as described previously [9]. Recording electrodes had resistances of 5–10 MΩ when filled with pipette solution, which for whole-cell recordings comprised (in mmol/l) 140 KCl, 5 $MgCl_2$, 3.8 $CaCl_2$, 10 EGTA, 10 HEPES, pH 7.2 (free [Ca^{2+}] of 100 nmol/l). For perforated-patch recordings, the electrode solution contained (in mmol/l): 140 KCl, 5 $MgCl_2$, 3.8 $CaCl_2$, 10 HEPES, 10 EGTA (pH 7.2) and 25–40 μg/ml amphotericin B (Sigma-Aldrich). After a minimum of 10 min of stable recording, normal saline containing altered glucose concentration and/or tolbutamide (100 μmol/l), diazoxide (250 μmol/l) (both Sigma-Aldrich) or NN414 (5 μmol/l; Novo Nordisk, Copenhagen, Denmark) was applied.

Statistical analysis Data are presented as means±SEM. Analysis of variance, one-sample *t* test and Student's paired or unpaired *t* tests were performed using GraphPad Prism (Prism 5) software (GraphPad Software, La Jolla, CA, USA). p values ≤0.05 were considered statistically significant.

Results

Expression of GT1-7 cell glucose transporter, hexokinase and functional K_{ATP} channel subunits GT1-7 cells show mRNA for the glucose transporters *Glut1*, *Glut3* and *Glut4*, but not *Glut2* (Fig. 1a) and for the monocarboxylate transporter, *Slc16a7* (data not shown). mRNAs for *Hk1* and *Hk2*, but not *Hk3* or *Gck*, could be distinguished (Fig. 1a). In further attempts to demonstrate *Gck* mRNA, PCR was performed using pancreas-specific *Gck* mRNA primers, and expression of this transcript was confirmed, with GCK

protein also detectable by immunoblot in GT1-7 cells (Fig 1b,c). Sulfonylurea receptor subunit *Sur1* mRNA was expressed, with *Sur2b* mRNA also present, along with the pore-forming K_{ATP} subunit $K_{ir}6.2$ (with protein also detectable by immunoblot; electronic supplementary material [ESM] Fig. 1), although no $K_{ir}6.1$ or *Sur2a* mRNA was demonstrable (Fig. 1d). Perforated-patch recordings revealed electrical activity in saline containing 10 or 2.5 mmol/l glucose, with no difference in firing rates or membrane potential (V_m) (10 mmol/l, V_m=−51.0±2.5 mV [n=8]; 2.5 mmol/l, V_m=−48.8±2.2 mV [n=5]; p>0.1). GT1-7 cells in 10 mmol/l glucose and challenged with 2.5 mmol/l glucose also showed no change in V_m or firing rate (data not shown). For cells in 2.5 mmol/l glucose, addition of tolbutamide (200 μmol/l) caused a modest depolarisation (<3 mV) and increased firing (Fig. 1e). In contrast, the K_{ATP} activator, diazoxide (250 μmol/l), or the SUR1-specific activator [17], NN414 (5 μmol/l), rapidly hyperpolarised V_m and inhibited firing (Fig. 1f,g). Whole-cell voltage clamp (Fig. 1h,i) showed significant K^+ conductance, after washout of cell ATP, which was blocked by tolbutamide (200 μmol/l). These results indicate the presence of functional K_{ATP} channels in GT1-7 cells, which are predominantly closed at euglycaemic (2.5 mmol/l) glucose.

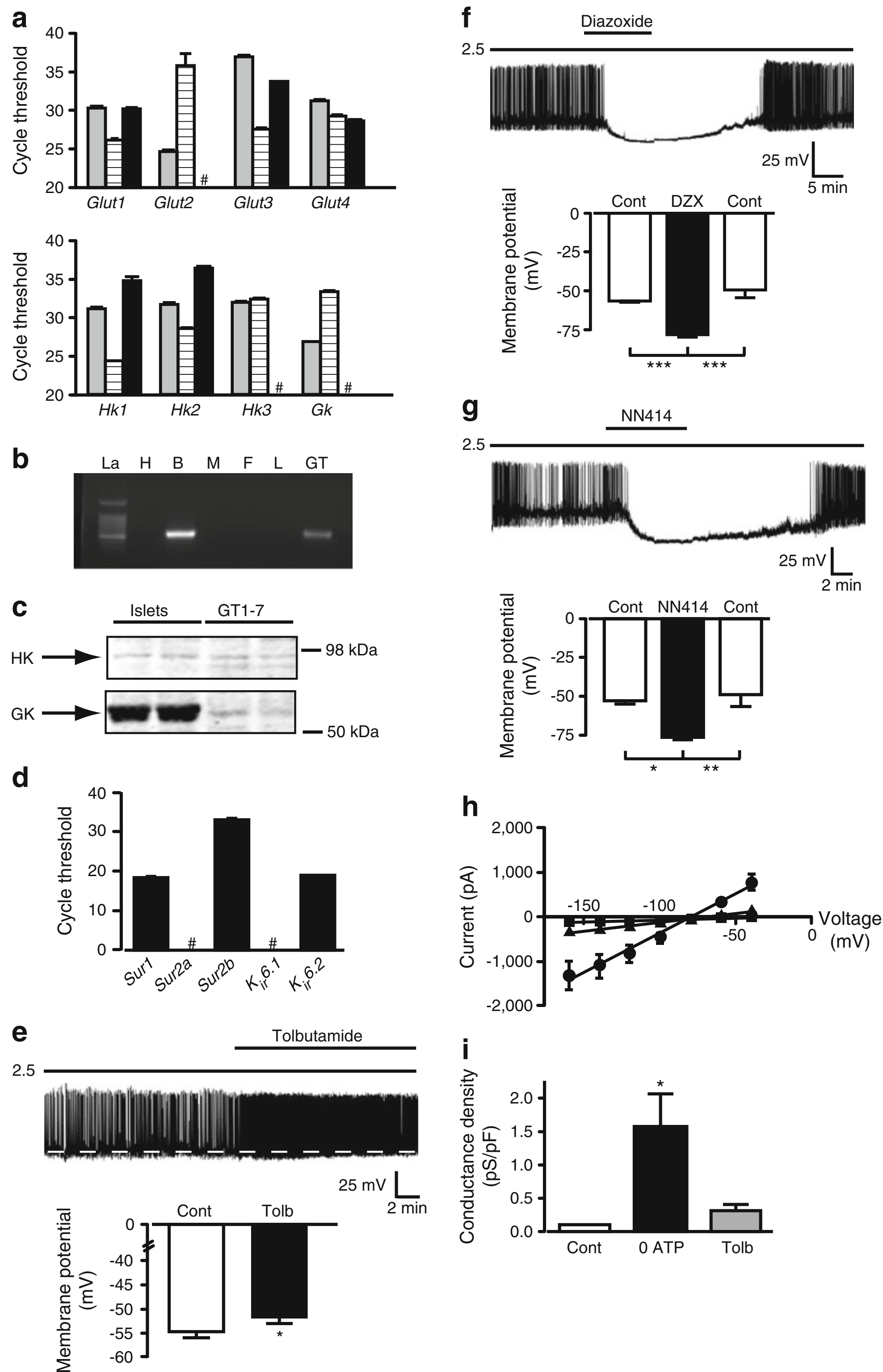
Hypothalamic GT1-7 cells sense brain glucose concentrations In contrast with the lack of sensitivity over the physiological plasma glucose range (10–2.5 mmol/l) GT1-7 cells responded, reversibly, to a lower glucose concentration (0.5 mmol/l) by hyperpolarisation and cessation of firing, which occurred independently of the initial glucose concentration (Fig. 2a,b). This sensitivity was observed regardless of the glucose concentration in the culture medium. Thus, for GT1-7 cells maintained in 2.5 mmol/l glucose/DMEM, followed by 2.5 mmol/l glucose/saline, a reduction to 0.5 mmol/l glucose caused reversible hyperpolarisation (2.5 mmol/l, V_m=−46.8±2.2 mV; 0.5 mmol/l, V_m=−61.3±1.7 mV; p<0.001, n=6) indistinguishable from cells maintained in high-glucose DMEM (Fig. 2a). GT1-7 cells responded to glucose concentrations below 1 mmol/l (Fig. 2c), in agreement with the glucose sensitivity reported for GE hypothalamic neurons [10]. To address glucose sensitivity further, we used another mechanism that monitors cell energy availability [18], AMPK, and examined phosphorylation of AMPK (p-AMPK) and its downstream effector, ACC (p-ACC). In GT1-7 cells exposed to 0.1 mmol/l glucose for 30 min and challenged with increasing glucose concentrations, maximal sensitivity occurred below 1 mmol/l (Fig. 2d and ESM Fig. 2). We also assessed AMPK phosphorylation in relation to hypoglycaemic glucose concentrations (2.5 mmol/l glucose starting point), which also demonstrates optimal sensing at concentrations below 0.5 mmol/l (Fig. 2e). As GT1-7 cells hyperpolarised

Fig. 1 GT1-7 cells express functional $K_{ir}6.2/Sur1$-containing K_{ATP} channels. (**a**) Bar graphs showing cycle threshold for real-time PCR amplification of *Glut1*, *2*, *3* and *4*, *Hk1*, *2* and *3* and *Gck* mRNA from liver (grey bars), brain (hatched bars) and GT1-7 cells (black bars) (n=3 for each). # represents non-detectable. (**b**) Detection of brain/pancreas-type GCK by PCR and immunoblot in GT1-7 cells. DNA was extracted from different tissues (H, heart; B, brain; M, skeletal muscle; F, fat; L, liver; GT, GT1-7 cell line). La, DNA ladder. (**c**) Representative immunoblots for hexokinase (HK) and GCK in GT1-7 cells in comparison with mouse islets. (**d**) Bar graphs showing cycle threshold for real-time PCR amplification of *Sur1*, *Sur2a*, *Sur2b*, $K_{ir}6.1$ and $K_{ir}6.2$ from GT1-7 cells (n=3 for each). (**e**) Perforated patch recording from GT1-7 cell showing excitation by tolbutamide (200 μmol/l). The bar graph shows mean values for membrane potential in 2.5 mmol/l glucose, in the absence (Cont) and presence (Tolb) of tolbutamide (n=6). (**f,g**) Perforated patch recordings from GT1-7 cells in 2.5 mmol/l glucose showing the reversible hyperpolarisation in response to diazoxide (DZX) (**f**) and NN414 (**g**). Bar graphs denote mean values of membrane potential in cells exposed to diazoxide (n=4) and NN414 (n=4). (**h**) Representative current–voltage relationships for voltage-clamped currents of GT1-7 cells. Mean currents were measured at various membrane potentials shortly after attaining whole-cell recording (i.e. before significant washout of ATP (control; squares) and 20 min later (after maximal washout of cellular ATP [0 ATP], circles) and with subsequent addition of tolbutamide (200 μmol/l, triangles). (**i**) Bar graph denotes mean conductance density (n=4) obtained under the recording conditions described in (**h**). Values are means±SEM. *p<0.05, **p<0.01, ***p<0.001

to 0.5 mmol/l glucose (Fig. 2a), we were concerned that the immunoblot method was insufficiently sensitive. Consequently, direct AMPK activity assay showed that glucose reduction from 2.5 to 0.5 mmol/l significantly increased total AMPK activity after 15 min (Fig. 2f), when neuronal hyperpolarisation is maximal. In conclusion, the glucose concentrations that engendered the largest change in AMPK activity were between 1.0 and 0.1 mmol/l, in good agreement with the electrical sensitivity to hypoglycaemia.

Glucose uptake and phosphorylation are required to maintain K_{ATP} closure in GT1-7 cells Expression studies showed mRNA for *Glut1*, *Glut3*, *Glut4* and hexokinase isoforms *Hk1*, *Hk2* and *Gck*. Cytochalasin B (20 μmol/l), an inhibitor of facilitated glucose transporters [19], rapidly caused hyperpolarisation by opening K_{ATP}, as denoted by tolbutamide reversal (Fig. 3a). The non-specific hexokinase inhibitor, alloxan (1 mmol/l), or replacement of glucose with the anti-metabolite, 2-deoxyglucose, also hyperpolarised GT1-7 cells (Fig. 3b,c). These results indicate that glucose uptake and metabolism are required to maintain the resting potential of GT1-7 hypothalamic neurons.

As *Gck* mRNA and protein abundance were low, we used an alternative approach to demonstrate that GCK contributed to glucose-sensing behaviour in GT1-7 cells. The GCK activator, GKA50, prevents hyperpolarisation of pancreatic beta cells in response to hypoglycaemic challenge [9] and increases insulin secretion [20]. After hyperpolarisation by 0.5 mmol/l glucose, application of GKA50 (1 μmol/l) caused depolarisation and increased firing (Fig. 3d),



indicating increased glucose metabolic flux. Central neurons also metabolise lactate if their glucose supply is restricted [21], thus we determined whether GT1-7 cells could use this alternative energy source to maintain V_m under hypoglycaemic conditions. GT1-7 cells exposed to 0.1 mmol/l glucose were depolarised when challenged with lactate at concentrations from 0.1 to 3.0 mmol/l (Fig. 3e). Although the mean changes in V_m induced by lactate only showed significance at 1.0 and 3.0 mmol/l, we observed cells that clearly responded to 0.3 mmol/l lactate (Fig. 3e).

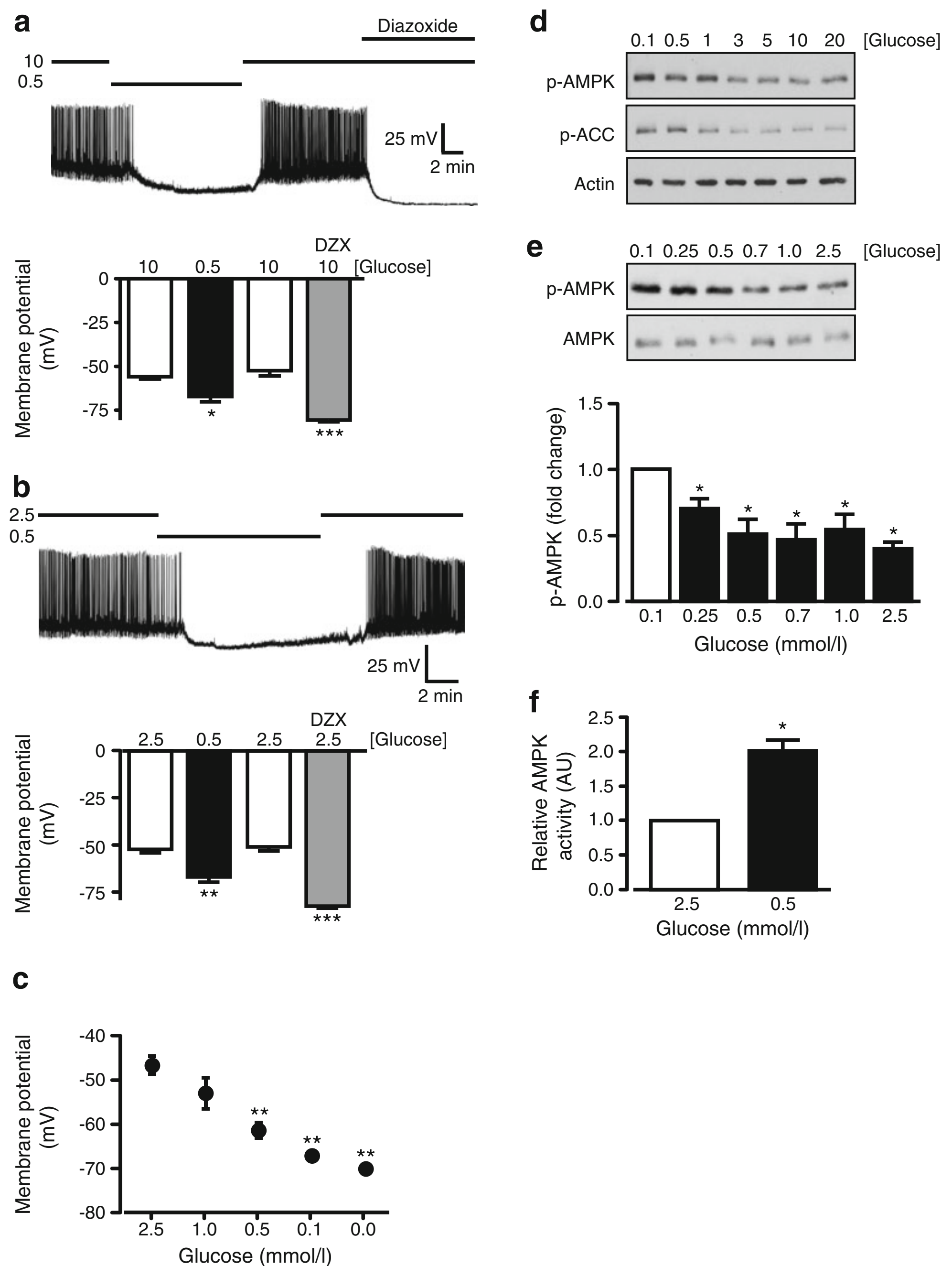


Fig. 2 GT1-7 cells are sensitive to brain glucose concentrations. (**a**,**b**) GT1-7 cells respond, reversibly, to a reduction in glucose from 10 (**a**) or 2.5 (**b**) to 0.5 mmol/l by hyperpolarisation and cessation of firing. Bar graphs show mean values for membrane potential of cells exposed to 10 (**a**; n=7) or 2.5 (**b**; n=5) mmol/l glucose, or 0.5 mmol/l glucose and diazoxide (DZX). (**c**) Mean membrane potential values for GT1-7 cells as a function of glucose concentration (n=5–7). (**d**) Representative immunoblot showing the effect of increasing glucose concentration (0.1–20 mmol/l) on p-AMPK and p-ACC levels. (**e**) Representative immunoblot showing the effect of glucose (0.1–2.5 mmol/l) on p-AMPK and total AMPK levels. Bar graph shows relative mean level of p-AMPK as a function of glucose concentration (n=6). (**f**) AMPK activity (arbitrary units [AU]) measured in GT1-7 cells after their exposure to 2.5 and 0.5 mmol/l glucose for 15 min (n=3). Values are means±SEM. *p<0.05, **p<0.01, ***p<0.001

Decreased AMPKα2, but not AMPKα1, activity diminishes the glucose sensitivity of GT1-7 cells The AMPKα2 subunit is required for hypoglycaemia sensing in pancreatic beta cells [9] and subpopulations of hypothalamic neurons [10]. Consequently, we examined whether this protein was also linked to glucose sensing in GT1-7 cells. To reduce AMPK levels and activity, GT1-7 cells were infected with lentivirus expressing shRNA to *Ampkα2* (sh*Ampkα2*), *Ampkα1* (sh*Ampkα1*) or a control, scrambled sequence (shCont). Immunoblots confirmed that both AMPK catalytic subunits were present, that treatment of GT1-7 cells with control vector had no effect on isoform protein levels, and that sh*Ampkα2* reduced AMPKα2, but not AMPKα1, protein levels (Fig. 4a). Measurement of AMPK isoform specific activity showed that GT1-7 cells exhibited predominantly AMPKα1 (0.395±0.068 mU min^{-1} mg^{-1}; n=17) over AMPKα2 (0.0077±0.0017 mU min^{-1} mg^{-1}; n=17) activity, and that 100 μmol/l H_2O_2 significantly raised the activity of both isoforms (Fig. 4b,d). Although sh*Ampkα2* treatment of GT1-7 cells did not significantly alter basal AMPKα2 activity (Fig. 4b,c), it did prevent H_2O_2 activation of AMPKα2, but not AMPKα1, activity (Fig. 4b,d) and importantly prevented stimulation of AMPKα2 activity by hypoglycaemia (Fig. 4c).

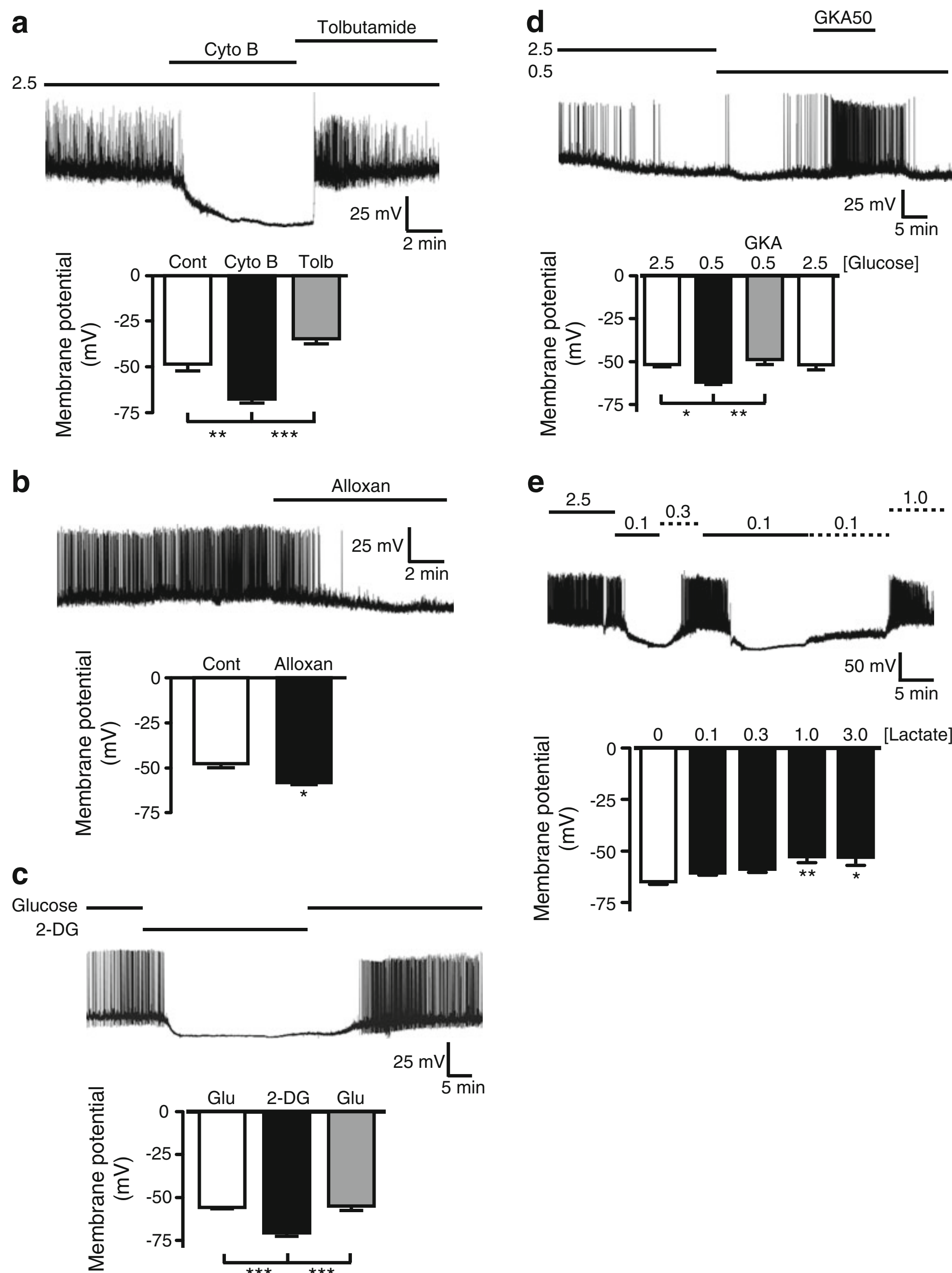


Fig. 3 Nutrient metabolism controls excitability of GT1-7 cells. (**a**) Perforated patch recording showing that cytochalasin B (Cyto B; 20 μmol/l) hyperpolarises GT1-7 cells in 2.5 mmol/l glucose, an action reversed by tolbutamide (200 μmol/l). Bar graph shows mean values for membrane potential of cells exposed to 2.5 mmol/l glucose (Cont), cytochalasin B and tolbutamide (Tolb; n=5). (**b**) Alloxan (1 mmol/l) hyperpolarises GT1-7 cells. Bar graph shows mean membrane potentials under control conditions (2.5 mmol/l glucose) and in alloxan (n=4). (**c**) Replacement of 2.5 mmol/l glucose with 2.5 mmol/l 2-deoxyglucose (2-DG) reversibly hyperpolarises GT1-7 cells. Bar graph denotes mean membrane potential of cells exposed to glucose and 2-deoxyglucose (n=4). (**d**) Application of the GCK activator, GKA50 (1 μmol/l) reverses the hyperpolarisation and inhibition of firing caused by 0.5 mmol/l glucose. Bar graph shows mean membrane potential of cells exposed to 2.5 mmol/l glucose and 0.5 mmol/l glucose with or without GKA50 (n=6). (**e**) Lactate compensates for low glucose in maintenance of GT1-7 cell membrane potential and excitability. Solid and broken lines denote glucose and lactate concentrations, respectively, with individual concentrations given above the trace. Bar graph shows mean membrane potential of cells in 0.1 mmol/l glucose (white bar) or lactate (0.1–3.0 mmol/l; n=5–9; black bars). Values are means±SEM. *p<0.05, **p<0.01, ***p<0.001

shCont-treated GT1-7 cells displayed electrical activity in 2.5 mmol/l glucose and responded to 0.5 mmol/l glucose by hyperpolarisation and cessation of firing (Fig. 4e) in a manner indistinguishable from untreated cells. In contrast,

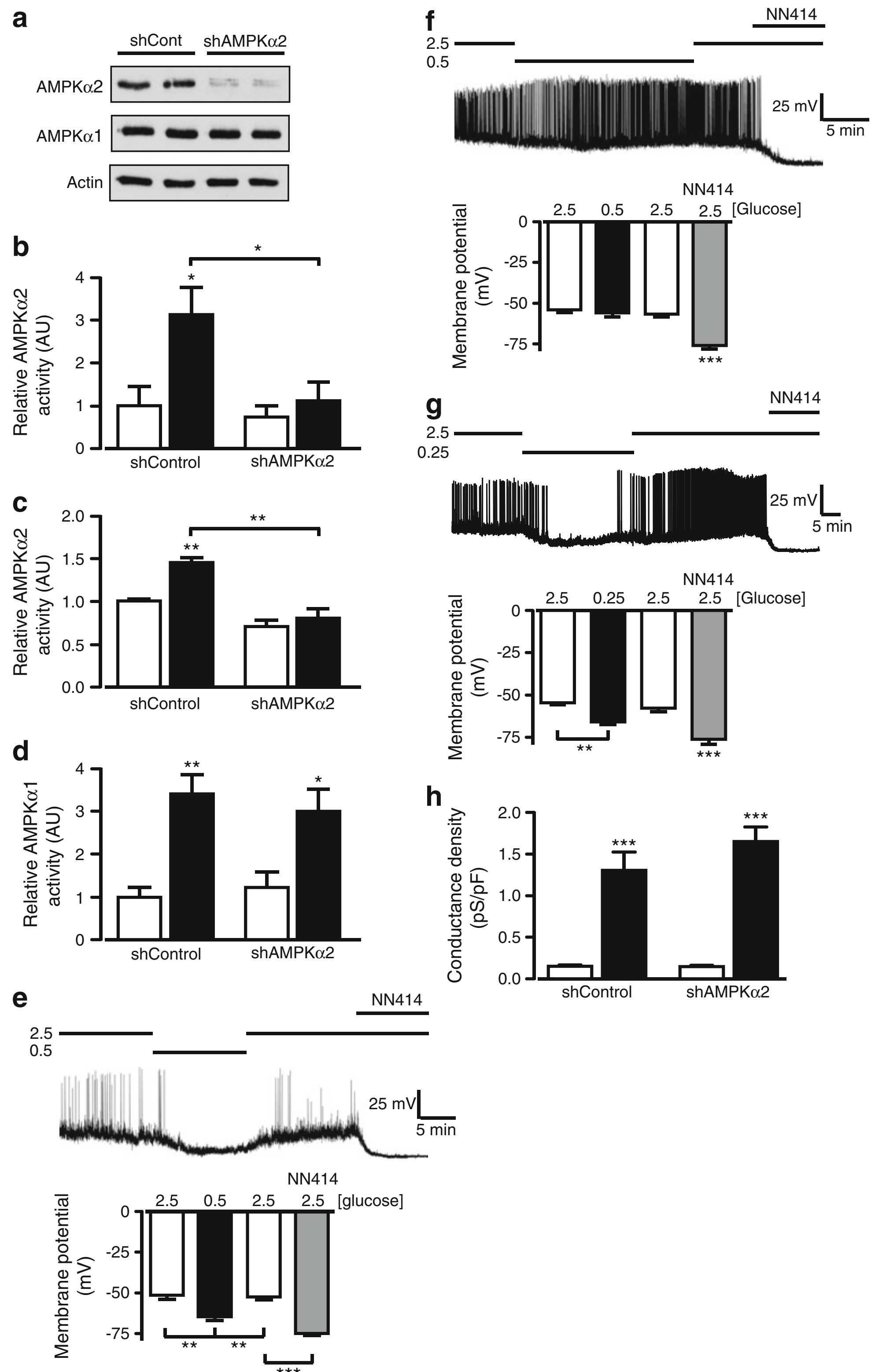


Fig. 4 AMPKα2 activity modifies GT1-7 sensitivity to hypoglycaemia. (**a**) Lentiviral delivery of shRNA targeting AMPKα2 reduces AMPKα2, but not AMPKα1, protein levels. Cells treated with control lentiviral vector (shCont) are unaffected. (**b**–**d**) sh*Ampkα2* prevents H_2O_2- (**b**) and low-glucose- (0.5 mmol/l; **c**) induced increase in AMPKα2 activity (arbitrary units [AU]), but has no effect (**d**) on H_2O_2-induced increase in AMPKα1 activity, (n=4–7). White bars denote vehicle-treated cells and black bars H_2O_2-treated cells in (**b**) and (**d**), whereas in (**c**) white bars denote cells exposed to 2.5 mmol/l glucose and black bars cells exposed to 0.5 mmol/l glucose. (**e**) Representative recording showing the electrical response of GT1-7 cells infected with shCont to a reduction in glucose from 2.5 to 0.5 mmol/l and to application of NN414. Bar graph shows mean values for membrane potentials under the conditions described (n=3–6). (**f**,**g**) GT1-7 cells infected with shAMPKα2 show no electrical response to 0.5 mmol/l glucose (**f**), but do respond to a more extreme hypoglycaemic (0.25 mmol/l glucose) stimulus (**g**). Bar graphs show mean values of membrane potential for shAMPKα2-treated GT1-7 cells challenged with 0.5 mmol/l (**f**; n=6) and 0.25 mmol/l (**g**; n=5) glucose. (**h**) Bar graph denotes mean conductance density under voltage clamp, in control (immediately after formation of stable clamp) and after a washout of ATP from the cell (20 min) for GT1-7 cells infected with shCont and shAMPKα2 lentivirus (n=6–8). Values are means±SEM. *p<0.05, **p<0.01, ***p<0.001

sh*Ampkα2*-treated cells were less responsive to hypoglycaemic challenge, with 0.5 mmol/l glucose having no effect on V_m or firing rate (Fig. 4f). However, these cells were responsive to more severe hypoglycaemic challenge, with 0.25 mmol/l (Fig. 4g) and 0.1 mmol/l (ΔV_{Cont}=−9.7±2.1 mV; $\Delta V_{shAMPK\alpha2}$=−13.5±4.3 mV; n=4; p>0.1) glucose exposure causing hyperpolarisation. Thus treatment of GT1-7 cells with sh*Ampkα2* shifts the threshold for

detection electrically to a more severe hypoglycaemia stimulus. This shift in glucose-sensing capability was not associated with any change in maximal K_{ATP} conductance of GT1-7 cells (Fig. 4h). As responsiveness to NN414 was also unaltered (compare Fig. 4e,f and Fig. 1g), it is likely that no change in K_{ATP} availability is associated with modification of glucose sensing. Sh*Ampkα1*-treated GT1-7 cells exhibited reduced AMPKα1 protein levels (Fig. 5a) and depressed H_2O_2-stimulated AMPKα1 activity (Fig. 5b), which was not associated with loss of AMPKα2 protein levels or activity. Furthermore, stimulation with H_2O_2 increased AMPKα2 activity, identical with the control (Fig. 5d). Although sh*Ampkα1* treatment of GT1-7 cells ablated stimulation of AMPKα1 activity by 0.5 mmol/l glucose (Fig. 5c), robust and reproducible hyperpolarising responses to 0.5 mmol/l glucose were observed (Fig. 5e), indicating that AMPKα2, rather than AMPKα1, activity is required for cells to respond electrically to hypoglycaemia.

UCP2 may also contribute to glucose sensing in GT1-7 cells Previous work suggests a role for UCP2 in the glucose-sensing behaviour of beta cells and hypothalamic neurons [22–24], with K_{ATP} activation and hyperpolarisation induced by low glucose in beta cells and proopiomelanocortin (POMC) neurons prevented by pharmacological inhibition of UCP2 with genipin [9, 24]. In agreement, we found that genipin (100 μmol/l) prevented GT1-7 cells from responding electrically to hypoglycaemia (Fig. 6a). Furthermore, treatment of GT1-7 cells with sh*Ampkα2* significantly reduced *Ucp2* mRNA levels, in comparison with shCont-treated cells (Fig. 6b), suggesting a close link between AMPKα2 activity and UCP2 content.

Discussion

GT1-7 cells, which make and secrete gonadotrophin-releasing hormone (GnRH) exhibit intrinsic glucose-sensing properties after reduction of extracellular glucose from the euglycaemic levels (2.5 mmol/l) normally associated with brain. As reported here for GT1-7 cells, mouse GnRH neurons express mRNA for *Gck* and the K_{ATP} subunits $K_{ir}6.2$ and *Sur1*, and respond to lowered glucose by K_{ATP}-dependent hyperpolarisation [25]. Furthermore, $K_{ir}6.2$ and *Sur1* mRNAs have been demonstrated in hypothalamic GE neurons using single-cell RT-PCR [7, 26]. However, the presence of these transcripts did not completely correlate with GE neuron phenotype, and expression of the combination, $K_{ir}6.1$ and *Sur1*, has also been reported in hypothalamic GE neurons [27]. Our findings that GT1-7 cells express

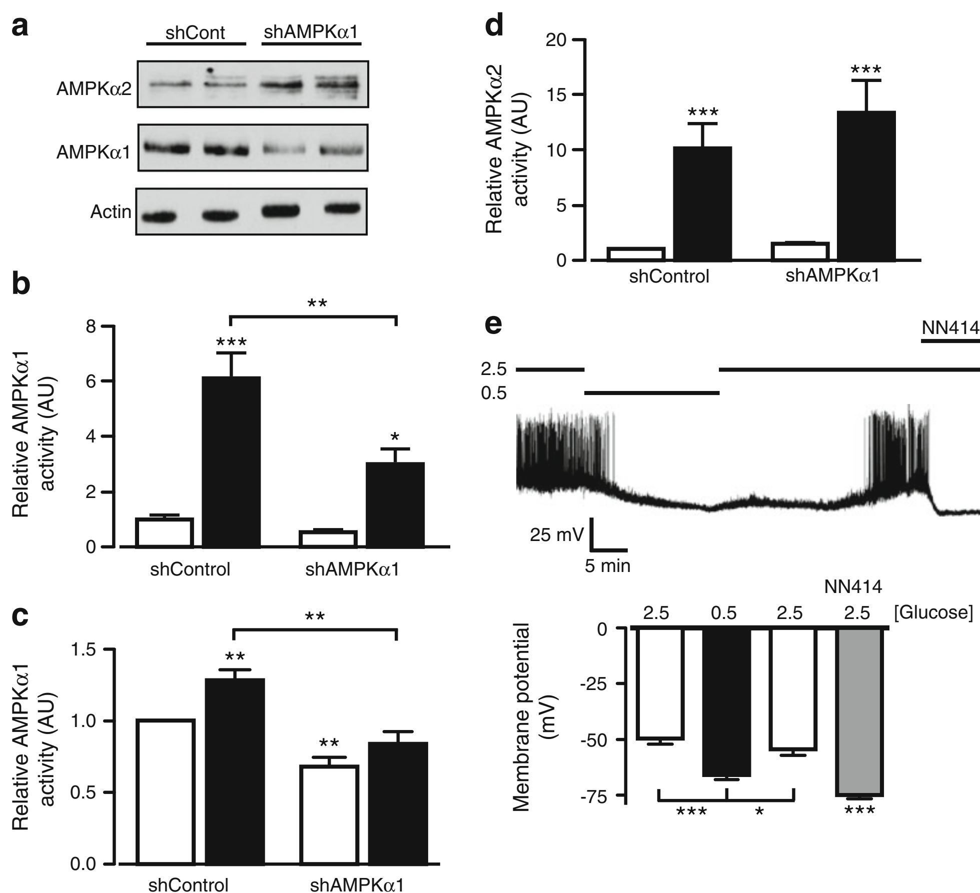


Fig. 5 Hypoglycaemic responses are insensitive to reduction in AMPKα1. (**a**) Lentiviral delivery of shRNA targeting AMPKα1 reduces AMPKα1, but not AMPKα2, protein levels. Cells treated with control lentiviral vector (shCont) are unaffected. (**b**–**d**) shAMPKα1 reduces H_2O_2- (**b**) and 0.5 mmol/l glucose (**c**)-induced increase in AMPKα1 activity (arbitrary units [AU]), but has no effect (**d**) on H_2O_2-induced increase in AMPKα2 activity (*n*=4–12). White bars denote vehicle-treated cells and black bars H_2O_2-treated cells in (**b**) and (**d**), whereas in (**c**) white bars denote cells exposed to 2.5 mmol/l glucose and black bars cells exposed to 0.5 mmol/l glucose. (**e**) GT1-7 cells infected with shAMPKα1 show a normal electrical response to 0.5 mmol/l glucose. Bar graph shows mean values of membrane potential for shAMPKα1-treated GT1-7 cells challenged with 0.5 mmol/l glucose and NN414 (*n*=4–6). Values are means±SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$

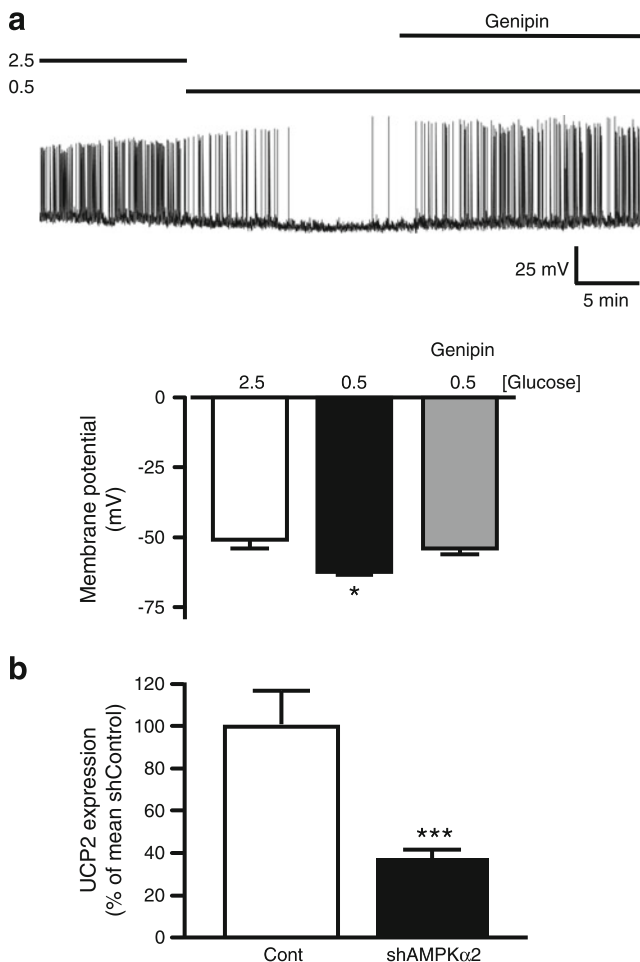


Fig. 6 Reduction in UCP2 may link AMPK and glucose sensing in GT1-7 cells. (**a**) Representative recording showing the effects of genipin (100 μmol/l) on uninfected GT1-7 cells, in the presence of 0.5 mmol/l glucose. Bar graph shows mean values of membrane potential for cells in 2.5 mmol/l glucose and 0.5 mmol/l glucose with or without genipin (n=5). (**b**) Treatment of cells with shAMPKα2 reduces UCP2 mRNA levels (n=15–17). Values are means±SEM. *p<0.05, ***p<0.001

$K_{ir}6.2$ and *Sur1*, but not *$K_{ir}6.1$*, mRNA support the notion that the beta cell K_{ATP} subunit combination underlies GE neuron effector responses to hypoglycaemia. Demonstrable levels of $K_{ir}6.2$ protein and K_{ATP} activation by diazoxide and NN414 and inhibition by tolbutamide also support this subunit permutation. In keeping with the proposed role for GCK as 'gatekeeper' of neuronal glucose sensing [28, 29], GCK inhibition by alloxan or activation by GKA50 [20] mimicked or reversed the effects of hypoglycaemic challenge. These results indicate major roles for K_{ATP} and GCK in mediating glucose sensing in GT1-7 cells as described for pancreatic beta cells [30, 31]. In rodent beta cells, GLUT2 mediates glucose entry under physiological glucose concentrations [32]. However, GT1-7 cells only expressed *Glut1*, *Glut3* and *Glut4* transcripts. GLUT2 is mainly located in astrocytes, with GLUT3 being the primary neuronal transporter, although GLUT1 and GLUT4 have been reported in neurons [33]. Indeed, glial GLUT2 may be required for normal glucagon secretion in response to hypoglycaemia [34]. GLUT1 and GLUT3 have low K_m values (~1 mmol/l [33]) consistent with the glucose sensitivity of hypothalamic GE neurons [10, 35, 36] and GT1-7 cells. GLUT4 has a K_m that encompasses the physiological range of brain glucose and could allow insulin-mediated modulation of glucose uptake, as described for hypothalamic GE neurons [37].

The lack of responsiveness to glucose above 2.5 mmol/l and the small effect of tolbutamide in 2.5 mmol/l glucose indicate that GT1-7 K_{ATP} channels are mostly closed in euglycaemic and hyperglycaemic conditions. This has previously been reported for GE neurons [38], and contrasts with larger beta cell responses to tolbutamide under euglycaemic conditions, indicating the greater influence of K_{ATP} on the resting V_m of beta cells. Indeed, the baseline resting V_m of GT1-7 cells of~−50 mV is similar to that reported previously for hypothalamic GE neurons [10, 26, 27, 36, 37, 39]. In contrast, reducing the glucose concentration to 1.0 mmol/l or below caused reversible K_{ATP}-dependent hyperpolarisation and reduction, or loss, of firing of GT1-7 cells. The sensitivity of GT1-7 V_m to agents that suppress glucose uptake and metabolism indicates the requirement, as observed for beta cells, for glucose entry and metabolism to maintain K_{ATP} in the predominantly closed conformation. Glial cells, such as astrocytes, provide neurons with energy substrates [3] such as lactate, which, in conjunction with monocarboxylate transporters, cause closure of K_{ATP} in GE neurons [39]. Thus astrocyte lactate production may act as an energy fuel reserve for neurons, maintaining their electrical activity during hypoglycaemia [1]. Although GT1-7 cells are intrinsic glucose sensors, the presence of *Slc16a7* mRNA and the ability of exogenous lactate to depolarise and excite these cells under conditions of glucose deprivation indicate that lactate conversion into pyruvate in neurons could maintain their V_m and excitability during hypoglycaemic episodes. Indeed, the similar concentration response of lactate and glucose on V_m indicates that lactate is a more effective energy substrate, on an energy basis, at closing K_{ATP} channels. Consequently, GT1-7 cells behave as direct glucose and lactate sensors.

AMPK is an important nutrient sensor and effector mechanism in cells, allowing detection of lowered cell energy status with coupling to intrinsic cell mechanisms designed to restore energy balance. Changes in AMPK activity have been implicated in counter-regulatory hormone responses to hypoglycaemia [11, 12], and a role for AMPK has been proposed for hypoglycaemia-dependent depolarisation of hypothalamic GI neurons [40, 41]. In addition, deletion of the AMPKα2 catalytic subunit in beta cells [9] and identified hypothalamic neurons [10] prevents hypoglycaemic challenge from K_{ATP} activation

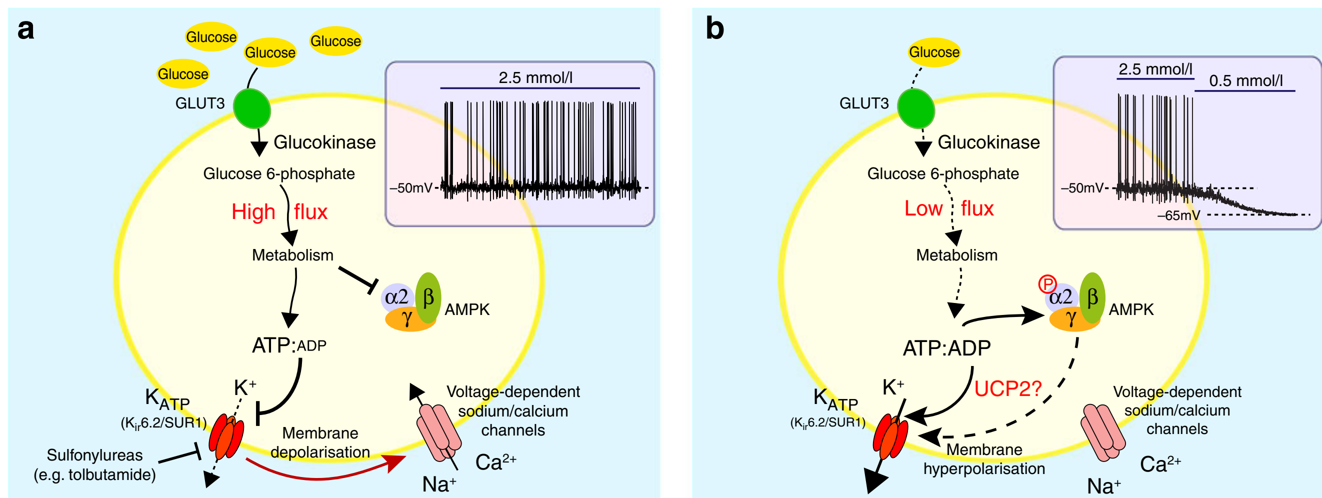


Fig. 7 Model of GT1-7 cell illustrating the molecular components indicative of a GE neuron. In order to correlate neuronal firing with glucose concentration, the cell must have a glucose-sensing and measuring system. This is denoted by the presence of the glucose-transport protein GLUT3 and glucokinase. (**a**) Glucose uptake and glucokinase activity are sufficient under euglycaemic conditions (2.5 mmol/l) to produce sufficient metabolic flux to maintain a high ATP/ADP ratio. This keeps AMPKα2 activity low (basal) and K_{ATP} channels predominantly in the closed conformation, thus maintaining a relatively depolarised membrane potential and regular firing (as illustrated by the inset figure). (**b**) If the cell is now exposed to a hypoglycaemic environment (e.g. 0.5 mmol/l glucose), there is reduced glucose metabolic flux, which results in a reduced ATP/ADP ratio (thought to be due to increased ADP, as phosphotransfer reactions maintain ATP levels). This rise in sub-plasma membrane ADP, in conjunction with increased AMPKα2 activity (possibly associated with increased UCP2 activity) causes increased K_{ATP} channel activity, resulting in cell hyperpolarisation and cessation of firing

and hyperpolarisation. Hypoglycaemia increases AMPK activity in GT1-7 cells with a glucose concentration-dependence that mirrors the electrical change. By using shRNA targeted to AMPKα subunits, we decreased protein levels of the targeted subunit sufficiently to reduce its maximal activation by H_2O_2 and prevent AMPKα activation by 0.5 mmol/l glucose. This demonstrated that reducing AMPKα2, but not AMPKα1, activity prevented the hyperpolarising response to 0.5 mmol/l glucose, but not to stronger stimuli, indicating a shift in the glucose-sensing threshold away from the physiologically relevant range. This result puts AMPKα2 activity (which is only ~2% of total AMPK activity in GT1-7 cells) directly in the glucose-sensing pathway of GE neurons.

Therefore, what links AMPKα2 activity with GE neuron metabolism and K_{ATP} channels? It is generally considered that modulation of beta cell K_{ATP} activity by glucose metabolism is driven by changes in the ATP/ADP ratio. Glucose sensing is negatively regulated by raised UCP2 activity, which is argued to diminish the yield of ATP from glucose, causing beta cell dysfunction [23, 42]. *Ucp2* is highly expressed in the hypothalamus [43], and recent studies suggest that UCP2 negatively regulates glucose sensing in hypothalamic neurons [24, 44]. Increased UCP2 decreases mitochondrial V_m and respiration, via uncoupling, and is expected to reduce the ATP/ADP ratio, thus causing K_{ATP} opening. This scenario is supported by the observation that genipin closes K_{ATP} and depolarises beta cells and POMC neurons, and this requires the presence of UCP2 [30, 32]. Under hypoglycaemic conditions, genipin closes K_{ATP} and depolarises GT1-7 cells, suggesting a UCP2-mediated change in glucose metabolism in these cells. However, we have been unable to detect an alteration in bulk ATP/ADP during hypoglycaemia in GT1-7 cells (ESM Fig. 3), consistent with a previous study [39] of GE neurons. It is plausible that localised, sub-membrane alterations of ATP/ADP are responsible for generating the signals that control K_{ATP} gating under hypoglycaemic conditions and our measures are simply insufficiently sensitive. Nevertheless, reducing AMPKα2 in GT1-7 cells significantly decreased *Ucp2* mRNA levels, an outcome also reported in islets from mice lacking AMPKα2 in beta cells [9]. Thus AMPKα2 activity may, in an as yet undefined way, be positively linked to *Ucp2* transcription in GE neurons and pancreatic beta cells. Interestingly, activation of AMPK increases *Ucp2* expression in beta cells [45–47].

Our findings demonstrate that the GT1-7 cell line is an excellent model with which to probe hypothalamic glucose-sensing mechanisms. GT1-7 cells replicate the critical features of hypothalamic GE neurons, with functional expression of the molecular components essential for metabolic sensing and transduction to an electrical signal, as exemplified by beta cells. Decreasing AMPKα2 activity in GT1-7 cells diminished electrical sensitivity to a moderate hypoglycaemic

stimulus, an action that may require reduced UCP2, although we have no definitive data to prove this at present. We hypothesise that AMPKα2 acts as a sensor of fluctuations in glucose concentration by connecting glucose metabolism, through modulation of UCP2, with changes in local nucleotide levels (e.g. ADP), K_{ATP} channel activity and electrical excitability (Fig. 7). Furthermore, it is possible that hormones such as leptin and/or amylin could, by modifying AMPK activity [48], alter this intrinsic glucose-sensing behaviour. Functional deficits of any one of the classical components of glucose-sensing cells (e.g. K_{ATP}, GLUT2 and GCK) engender dysfunctional output, as exemplified for pancreatic beta cells and type 2 diabetes [30, 49, 50]. Consequently any loss of function involving AMPKα2–UCP2 in beta cells or GE neurons may have a similar outcome. Therefore, in the context of hypothalamic glucose sensing, it will be interesting to determine whether defective glucose counter-regulation after recurrent bouts of hypoglycaemia is associated with alterations in the putative hypothalamic AMPKα2–UCP2 pathway.

Acknowledgements We thank Novo Nordisk for providing NN414.

Funding This study was funded by: grants from the Wellcome Trust (grant numbers 068692 and 086989) and Diabetes UK (grant number RD08/0003681) to M.L.J. Ashford; a Juvenile Diabetes Research Foundation (JDRF) Postdoctoral Fellowship to C. Beall (grant number 3-576-2010); grants from JDRF and European Foundation for the study of Diabetes to R.J. McCrimmon, and from the British Heart Foundation to A. Jovanović.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript. Novo Nordisk had no involvement in this study.

Contribution statement CB and DLH acquired and analysed data, contributed to experimental design, interpretation of data and to revision of the manuscript, JG, LL, KW, MPS, SD, FBA, EH, QD and AJ acquired and analysed data and revised the manuscript, RJM contributed to conception of study, experimental design, interpretation of data and revised the manuscript; MLJA contributed to conception of study, experimental design, interpretation of data, and drafted, revised and edited the manuscript. All authors approved the final version.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. McCrimmon RJ, Sherwin RS (2010) Hypoglycemia in type 1 diabetes. Diabetes 59:2333–2339
2. Dunn-Meynell AA, Sanders NM, Compton D et al (2009) Relationship among brain and blood glucose levels and spontaneous and glucoprivic feeding. J Neurosci 29:7015–7022
3. Levin BE, Magnan C, Dunn-Meynell A, Le Foll C (2011) Metabolic sensing and the brain: who, what, where and how? Endocrinology 152:2552–2557
4. Karnani M, Burdakov D (2011) Multiple hypothalamic circuits sense and regulate glucose levels. Am J Physiol Regul Integr Comp Physiol 300:R47–R55
5. Tarasov A, Dusonchet J, Ashcroft FM (2004) Metabolic regulation of the pancreatic beta-cell ATP-sensitive K^+ channel: a pas de deux. Diabetes 53:S113–S122
6. Burdakov D, Luckman SM, Verkhratsky A (2005) Glucose-sensing neurons of the hypothalamus. Phil Trans R Soc B 360:2227–2235
7. Kang L, Routh VH, Kuzhikandathil EV, Gaspers LD, Levin BE (2004) Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. Diabetes 53:549–559
8. Gonzàlez JA, Reimann F, Burdakov D (2009) Dissociation between sensing and metabolism of glucose in sugar sensing neurones. J Physiol 585:41–48
9. Beall C, Piipari K, Al-Qassab H et al (2010) Loss of AMP-activated protein kinase alpha2 subunit in mouse beta-cells impairs glucose-stimulated insulin secretion and inhibits their sensitivity to hypoglycaemia. Biochem J 429:323–333
10. Claret M, Smith MA, Batterham RL et al (2007) AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. J Clin Invest 117:2325–2336
11. McCrimmon RJ, Shaw M, Fan X et al (2008) Key role for AMP-activated protein kinase in the ventromedial hypothalamus in regulating counterregulatory hormone responses to acute hypoglycemia. Diabetes 57:444–450
12. McCrimmon RJ, Fan X, Cheng H et al (2006) Activation of AMP-activated protein kinase within the ventromedial hypothalamus amplifies counterregulatory hormone responses in rats with defective counterregulation. Diabetes 55:1755–1760
13. Mellon PL, Windle JJ, Goldsmith PC, Padula CA, Roberts JL, Weiner RI (1990) Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. Neuron 5:1–10
14. Mirshamsi S, Laidlaw HA, Ning K et al (2004) Leptin and insulin stimulation of signalling pathways in arcuate nucleus neurones: PI 3-kinase dependent actin reorganization and K_{ATP} channel activation. BMC Neurosci 5:54
15. Hawley SA, Ross FA, Chevtzoff C et al (2010) Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. Cell Metab 11:554–565
16. Choudhury AI, Heffron H, Smith MA et al (2005) The role of insulin receptor substrate 2 in hypothalamic and ß-cell function. J Clin Invest 115:940–950
17. Dabrowski M, Larsen T, Ashcroft FM, Bondo Hansen J, Wahl P (2003) Potent and selective activation of the pancreatic beta-cell type K(ATP) channel by two novel diazoxide analogues. Diabetologia 46:1375–1382
18. Hardie DG (2011) Sensing of energy and nutrients by AMP-activated protein kinase. Am J Clin Nutr 93:891S–896S
19. Hara M, Matsuda Y, Hirai K, Okumura N, Nakagawa H (1989) Characteristics of glucose transport in neuronal cells and astrocytes from rat brain in primary culture. J Neurochem 52:902–908
20. Johnson D, Shepherd RM, Gill D, Gorman T, Smith DM, Dunne MJ (2007) Glucose-dependent modulation of insulin secretion and intracellular calcium ions by GKA50, a glucokinase activator. Diabetes 56:1694–1702
21. Pellerin L (2010) Food for thought: the importance of glucose and other energy substrates for sustaining brain function under varying levels of activity. Diabetes Metab 36:S59–S63
22. Zhang C-Y, Parton LE, Ye CP et al (2006) Genipin inhibits UCP2-mediated proton leak and acutely reverses obesity- and high

glucose-induced ß-cell dysfunction in isolated pancreatic islets. Cell Metab 3:417–427

23. Krauss S, Zhang C-Y, Scorrano L et al (2003) Superoxide-mediated activation of uncoupling protein 2 causes pancreatic ß-cell dysfunction. J Clin Invest 112:1831–1842
24. Parton LE, Ye CP, Coppari R et al (2007) Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. Nature 449:228–232
25. Zhang C, Bosch MA, Levine JE, Rønnekleiv OK, Kelly MJ (2007) Gonadotrophin-releasing hormone neurons express K(ATP) channels that are regulated by estrogen and responsive to glucose and metabolic inhibition. J Neurosci 27:10153–10164
26. Ibrahim N, Bosch MA, Smart JL et al (2003) Hypothalamic proopiomelanocortin neurons are glucose responsive and express K(ATP) channels. Endocrinology 144:1331–1340
27. Lee K, Dixon AK, Richardson PJ, Pinnock RD (1999) Glucose-receptive neurones in the rat ventromedial hypothalamus express KATP channels composed of Kir6.1 and SUR1 subunits. J Physiol 515:439–452
28. Yang XJ, Kow LM, Pfaff DW, Mobbs CV (2004) Metabolic pathways that mediate inhibition of hypothalamic neurons by glucose. Diabetes 53:67–73
29. Dunn-Meynell AA, Routh VH, Kang L, Gaspers L, Levin BE (2002) Glucokinase is the likely mediator of glucosensing in both glucose-excited and glucose-inhibited central neurons. Diabetes 51:2056–2065
30. McTaggart JS, Clark RH, Ashcroft FM (2010) The role of the KATP channel in glucose homeostasis in health and disease: more than meets the islet. J Physiol 588:3201–3209
31. Matchinsky FM (2002) Regulation of pancreatic beta-cell glucokinase: from basics to therapeutics. Diabetes 51:S394–S404
32. Thorens B (2001) GLUT2 in pancreatic and extra-pancreatic gluco-detection (review). Mol Membr Biol 18:265–273
33. Thorens B, Mueckler M (2010) Glucose transporters in the 21st century. Am J Physiol Endocrinol Metab 298:E141–E145
34. Marty N, Dallaporta M, Foretz M et al (2005) Regulation of glucagon secretion by glucose transporter type 2 (glut2) and astrocyte-dependent glucose sensors. J Clin Invest 115:545–3553
35. Wang R, Liu X, Hentges ST et al (2004) The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. Diabetes 53:1959–1965
36. Song Z, Levin B, McArdle JJ, Bakhos N, Routh VH (2001) Convergence of pre- and postsynaptic influences on glucosensing neurons in the ventromedial hypothalamic nucleus. Diabetes 50:2673–2681
37. Cotero VE, Routh VH (2009) Insulin blunts the response of glucose-excited neurons in the ventrolateral-ventromedial hypothalamic nucleus to decreased glucose. Am J Physiol Endocrinol Metab 296:E1101–E1109
38. Song Z, Routh VH (2005) Differential effects of glucose and lactate on glucosensing neurons in the ventromedial hypothalamus. Diabetes 54:15–22
39. Ainscow EK, Mirshamsi S, Tang T, Ashford ML, Rutter GA (2002) Dynamic imaging of free cytosolic ATP concentration during fuel sensing by rat hypothalamic neurones: evidence for ATP-independent control of ATP-sensitive K+channels. J Physiol 544:429–445
40. Mountjoy PD, Bailey SJ, Rutter GA (2007) Inhibition by glucose or leptin of hypothalamic neurons expressing neuropeptide Y requires changes in AMP-activated protein kinase activity. Diabetologia 50:168–177
41. Murphy BA, Fakira KA, Song Z, Beuve A, Routh VH (2009) AMP-activated protein kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-inhibited neurons. Am J Physiol Cell Physiol 297:C750–C758
42. Zhang C-Y, Baffy G, Perret P et al (2001) Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, ß-cell dysfunction and type 2 diabetes. Cell 105:745–755
43. Richard D, Clavel S, Huang Q, Sanchis D, Ricquier D (2001) Uncoupling protein 2 in the brain: distribution and function. Biochem Soc Trans 29:812–817
44. Kong D, Vong L, Parton LE et al (2010) Glucose stimulation of hypothalamic MCH neurons involves K(ATP) channels, is modulated by UCP2, and regulates peripheral glucose homeostasis. Cell Metab 12:545–552
45. Li LX, Skorpen F, Egeberg K, Jørgensen IH, Grill V (2002) Induction of uncoupling protein 2 mRNA in beta-cells is stimulated by oxidation of fatty acids but not by nutrient oversupply. Endocrinology 143:1371–1377
46. Wang Y, Nishi M, Doi A et al (2010) Ghrelin inhibits insulin secretion through AMPK-UCP2 pathway in beta cells. FEBS Lett 584:1503–1508
47. Calegari VC, Zoppi CC, Rezende LF, Silveira LR, Carneiro EM, Boschero AC (2011) Endurance training activates AMP-activated protein kinase, increases expression of uncoupling protein 2 and reduces insulin secretion from rat pancreatic islets. J Endocrinol 208:257–264
48. Moon H-S, Chamberland JP, Mantzoros CS (2012) Amylin and leptin activate overlapping signalling pathways in an additive manner in mouse GT1-7 hypothalamic, C2C12 muscle and AML12 liver cell lines. Diabetologia 55:215–225
49. Gloyn AL (2003) Glucokinase (GCK) mutations in hyper- and hypoglycemia: maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. Hum Mutat 22:353–362
50. Cerf ME (2007) High fat diet modulation of glucose sensing in the beta-cell. Med Sci Monit 13:RA12–17