Expression of GLP1-receptors in Insulin Containing Interneurons of the Cerebral Cortex of the Rat

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Abstract

(1) Aims/hypothesis. Glucagon-like peptide 1 (GLP1) receptors are expressed by pancreatic beta cells and promote insulin secretion. GLP1 receptor agonists have neural effects and are therapeutically promising against mild cognitive impairment and Alzheimer's disease. Our recent results showed that insulin is released by neurogliaform neurons in the cerebral cortex, but the expression of GLP1 receptors on insulin producing neocortical neurons has not been tested. In this study, we aim to determine whether GLP1 receptors are present in insulin containing neurons.

(2) Methods. We harvested the cytoplasm of electrophysiologically and anatomically identified neurogliaform interneurons during patch clamp recordings performed in slices of rat neocortex. Using single cell digital PCR, we determined copy numbers of the *Glp1r* mRNAs and other key genes in neurogliaform cells harvested in conditions corresponding to hypo- (0.5 mM) and hyperglycemia (10 mM). In addition, we performed whole cell patch clamp recordings on neurogliaform cells to test the effect of GLP1 receptor agonists for functional validation of single cell digital PCR results.

(3) Results. Single cell digital PCR revealed GLP1 receptor expression in neurogliaform cells and showed that copy numbers of mRNAs of the *Glp1r* gene in hyperglycemia exceeded that of hypoglycemic copy numbers by 9.6 times ($P<0.008$). Moreover, single cell digital PCR confirmed co-expression of *Glp1r* and *Ins2* mRNAs in neurogliaform cells. Functional expression of GLP1 receptors was confirmed with whole cell patch clamp electrophysiology showing reversible effect of GLP1 on neurogliaform cells. In addition, single cell digital PCR of neurogliaform cells revealed expression of transcription factors (*Pdx, Isl1, Mafb*) important in beta cell development.

(4) Conclusions/interpretation. Our results provide evidence for functional expression of GLP1 receptors in neurons known to release insulin in the cerebral cortex. Hyperglycemia increases the expression of GLP1 receptors in neurogliaform cells suggesting that endogenous incretins and therapeutic GLP1 receptor agonists might have effects on these neurons similar to that of pancreatic beta cells.

Key words:basic science, animal-rat, hormone receptors, gastro-entero pancreatic factors, other techniques.

Abbreviations: BETA2, basic helix-loop-helix transcription factor; GLP1, Glucagon-like peptide 1; TIDMS, type I diabetes mellitus signalling; T2DM, type 2 diabetes mellitus.

Research in context

- GLP1 action on pancreatic beta cells lead to insulin secretion and current treatment of type 2 diabetes mellitus include GLP1 receptor agonists as therapeutic agents
- Insulin and GLP1 delivered to the brain is therapeutically promising against mild cognitive impairment and Alzheimer's disease
- Insulin is synthesized by neurons of the cerebral cortex, but it remains a question whether GLP1 or therapeutically applied GLP1 receptor agonists find targets on neurons capable of insulin production
- We show GLP1 receptor and insulin co-expression in neurogliaform interneurons in the cerebral cortex
- The expression of GLP1 receptors in neurogliaform cells is modulated by extracellular glucose concentrations
- Functional expression of GLP1 receptors is supported by the reversible effect of GLP1 on neurogliaform cells
- Future therapeutic strategies might include modulation of neural insulin production in the brain by GLP-1 agonists for counteracting diabetes, obesity and neurodegenerative diseases

Introduction

Glucagon-like peptide 1 (GLP1) produced by L-cells of the intestine is important in blood glucose homeostasis acting through several classic mechanisms including the inhibition of gastric emptying, suppressing pancreatic glucagon secretion and enhancing insulin release in the pancreas[1]. Direct action of circulating GLP1 on G-protein coupled GLP1 receptors located on pancreatic beta cells leads to glucose-dependent closure of ATP-sensitive K^+ channelswith subsequent depolarization and Ca^{2+} influx and Ca^{2+} dependent release of Ca^{2+} from intra-cellular Ca^{2+} stores resulting in Ca^{2+}-dependent insulin secretion[1].It is of high clinical importance that GLP-1 reduces the concentrations of blood glucose only postprandially, when blood glucose levels exceed fasting concentrations[2]. Such glucose-dependent action renders intravenously administered GLP1 ineffective in producing hypoglycaemia and, consequently, current treatment of type 2 diabetes mellitus (T2DM) include GLP1 receptor agonists as therapeutic agents.

Circulating GLP1 finds additional targets linked to insulin synthesis outside the pancreas. Native GLP-1 crosses the blood brain barrier [1, 3] and thus incretins arriving from the periphery, includingGLP-

1produced by intestinal L-cells and GLP1analogues prescribed in T2DM [4], have the possibility to act on neurons of the hippocampus and the neocortex known to express GLP1 receptors [5, 6]. In addition, neurons in the brain might also receive GLP1 from central sources according to results showing GLP1 expression in neurons located in the nucleus of the solitary tract in the brainstem[7, 8]. On the other hand, accumulating evidence based on experiments performed mostly in the rat shows that insulin is synthesized by neurons of the cerebral cortex[9–13]. Neuron-derived insulin was shown to be effective in regulating synaptic and microcircuit activity in the rat neocortex [13] and was suggested to regulate on demand energy homeostasis of neural networks[14].

Insulin delivered intranasally to the brain is therapeutically promising against mild cognitive impairment and Alzheimer's disease [15]and the GLP1 analogues used in diabetes treatment have preventive effects at the early stage of AD development[16, 17] Parkinson's disease[16, 18]and traumatic brain injury [19, 20]. It has been recently hypothesized that novel therapeutic strategies might include modulation of neural insulin production in the brain by GLP-1 agonists for counteracting diabetes, obesity and neurodegenerative diseases [14]. Therefore, it is of potential importance whether GLP1 of intestinal or neural origin or therapeutically applied GLP1 receptor agonists find targets on neurons capable of insulin production. We tested whether neurogliaform cells of the rat neocortex shown to release insulin locally the brain [13] have the molecular background to be involved in GLP1 signalling.

Methods

Electrophysiology

All procedures were performed with the approval of the University of Szeged (no. I-74-8/2016) and in accordance with the Guide for the care and use of laboratory animals, Eighth edition (2011) (http://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf). Male wistar rats (n = 106, P28-35, 190-220 g, weekly supplied by Charles River) were kept in individually ventilated cages (0.25 m^2) with biological bedding and ad libitum dry food and water. Animalswere anaesthetized by intraperitoneal injection of ketamine (30mg/kg) and xylazine (10mg/kg), and following the check of the cessation of pain reflexes and decapitation, coronal slices (350μm) were prepared from the somatosensory cortex. Animals used for this study also provided brain slices for other projects performed in parallel in the laboratory. Slice preparation and recordings were performed as described [13, 21]. Recordings were obtained at ~36 ˚C from cells visualized in layers I-III by infrared differential interference contrast videomicroscopy at depths 60-130 μm from the surface of the slice. Micropipettes

(5-7 MΩ) were filled with an intracellular solution containing 126 mM K-gluconate, 4 mMKCl, 10 mM HEPES, 10 mMcreatine phosphate, 8 mMbiocytin; pH 7.25; 300 mOsm supplemented with RNase Inhibitor (1U/µl, Life Technologies) to prevent any RNA degradation. Slices were preincubated in 0.5 mM glucose for 4 hours prior to hypoglycemicrecording sessions. Access resistance was monitored with -10 mV voltage steps in between experimental epochs and neurons were excluded from data analysis if access resistance exceeded 35 MΩ. Signals were filtered at 8 kHz, digitized at 16 kHz and analyzed with PULSE software. Traces shown are single sweeps for firing patterns.

Single cell reverse transcription and digital PCR

At the end of electrophysiological recordings, the intracellular content was aspirated into the recording pipettes by application of a gentle negative pressure while maintaining the tight seal [13, 21]. Pipettes were then delicately removed to allow outside-out patch formation, and the content of the pipettes (~ 1.5 µl) was expelled into a low-adsorbtion test tube (Axygen) containing 0.5 µl SingleCellProtect™ (Avidin Ltd. Szeged, Hungary) solution in order to prevent nucleic acid degradation and to be compatible with direct reverse transcription reaction. Samples were snap-frozen in liquid nitrogen and stored or immediately used for reverse transcription. The reverse transcription of the harvested cytoplasm was carried out in two steps. The first step was done for 5 min at 65 °C in a total reaction volume of 5 µl containing 2 µl intracellular solution and SingleCellProtect™ mix with the cytoplasmic contents of the neuron, 0.3 µlTaqMan Assays, 0.3 µl 10 mM dNTPs, 1 µl 5X first-strand buffer, 0.3 µl 0.1 mol/L DTT, 0.3 µl RNase inhibitor (Life Technologies) and 100 U of reverse transcriptase (Superscript III, Invitrogen). The second step of the reaction was carried out at 55 °C for 1 hour and then the reaction was stopped by heating at 75 °C for 15 min. The reverse transcription reaction mix was stored at -20 °C until PCR amplification.

For digital PCR analysis, half of the reverse transcription reaction mixture (2.5 µl), 2 µlTaqMan Assays (Life Technologies), 10 µlOpenArray Digital PCR Master Mix (Life Technologies) and nuclease free water (5.5 µl) were mixed in a total volume of 20 µl. Processing of the OpenArray slide, cycling in the OpenArray NT cycler and data analysis were done as previously described [13, 21]. For our dPCR protocol amplification, reactions with *CT* confidence values below 100 as well as reactions having *CT* values less than 23 or greater than 33 were considered primer dimers or background signals, respectively, and excluded from the data set.

Histology and reconstruction of neurons

Following electrophysiological recordings, slices were immersed in a fixative containing 4% paraformaldehyde, 15% (v/v) saturated picric acid and 1.25% glutaraldehyde in 0.1 M phosphate buffer (PB; pH=7.4) at 4˚C for at least 12 h. After several washes with 0.1 M PB, slices were frozen in liquid nitrogen then thawed in 0.1 M PB, embedded in 10% gelatine and further sectioned into 60 µm slices. Sections were incubated in a solution of conjugated avidin-biotin horseradish peroxidase (ABC; 1:100; Vector Labs) in Tris-buffered saline (TBS, pH=7.4) at 4˚C overnight. The enzyme reaction was revealed by 3'3-diaminobenzidine tetra-hydrochloride (0.05%) as chromogen and 0.01% H_2O_2 as oxidant. Sections were post fixed with 1% OsO_4 in 0.1 M PB. After several washes in distilled water, sections were stained in 1% uranyl acetate, dehydrated in ascending series of ethanol. Sections were infiltrated with epoxy resin (Durcupan, Sigma-Aldrich) overnight and embedded on glass slides. Three dimensional light microscopic reconstructions were carried out using Neurolucida system (MicroBrightField) with 100x objective.

Statistical analysis

Data are given as mean±S.D., datasets were statistically compared using one-way ANOVA, Kruskal-Wallis or Wilcoxon test and Mann Whitney U-test was used for electrophysiological measurements with SPSS software (IBM), differences were accepted as significant if $P<0.05$.

Results

Morpho-physiological characteristics of identified neurogliaform cells in hyper- and hypoglycaemia

We searched for interneurons showing characteristics of neurogliaform cells in layers 1 to 3 using the whole cell patch clamp mode of brain slices prepared from the somatosensory cortex of male rats (P28-35). Acute brain slices were maintained in artificial cerebrospinal fluid (ACSF) containing glucose either in the concentration used as standard for in vitro brain slices (10 mM, hyperglycaemia) or a concentration of 0.5 mM determined as hypoglycaemic external glucose concentration in the rat brain [22], but still suitable for whole cell recordings from interneurons [13]. Differential interference contrast microscopy was used to select putative interneurons based on perisomatic morphology and the identity of neurogliaform cells was first confirmed according to their late spiking firing characteristics in response to depolarizing current pulses[13](Fig. 1a). The use of biocytin in the patch clamp recording pipettes

allowed us to recover the morphology of the recorded cells and the identity of each neurogliaform cell included in this study (n = 87) was additionally confirmed by post hoc anatomical assessment of axonal morphology (Fig. 1a). Quantitative morphological analysis is beyond the scope of this study, however, the extremely dense axonal arborisation with small and frequently spaced boutons, the hallmark of neurogliaform cells[13]could be readily observed in samples recorded and biocytin filled in hyper- and hypoglycaemic conditions. In addition, no somatodendritic differences seemed to emerge between the two experimental groups and no morphological features considered pathological were observed.

We compared basic electrophysiological properties of neurogliaform cells recorded in 10 mM (n = 10) and 0.5 mM (n = 10) external glucose concentrations (Fig. 1b). Hyper- versus hypoglycaemic conditions had no significant effect on the resting membrane potential (-67.08±2.66 vs.-69.52±5.28 mV, P = 0.35, Mann-Whitney U-test), input resistance (113.15±34.03 vs. 85.82±17.91 mΩ, P = 0.079), amplitude of action potentials (75.76±6.84 vs. 72.30±6.1 mV, P = 0.39), interspike interval (0.103±0.030 vs. 0.078±0.033, ms, P = 0.094), half width of action potentials (0.82±0.27 vs. 0.56±0.12 ms, P = 0.079), action potential threshold (-30.89±1.77 vs. -30.9±4.8 mV, P = 0.71) and action potential accommodation (131.86±56.51 vs. 155.25±43.51 %, P = 0.15). However, significant differences emerged between neurogliaform cells recorded in 10 versus 0.5 mM glucose when measuring accommodation in the amplitudes of successive action potentials (90.04±6.18 vs. 81.44±6.47 %, P = 0.010) and accommodation in the half widths of successive action potentials (129.73±10.75 vs. 145.53±12.96 %, P = 0.019). Thus, apart from minor differences possibly due to the relatively lower metabolic supply in hypoglycaemia slightly affecting the amplitude and duration of action potentials during sustained activity, electrophysiological and morphological features of neurogliaform cells appear stable in our experimental conditions.

Functional expression of GLP1 receptors and related molecular characteristics of identified neurogliaform cells

Previous experiments suggest modulation of the *Ins2* gene in neurogliaform cells in response to changes in extracellular glucose concentration [13] indicating that the function of these neurons of the cerebral cortex might have partially similar molecular and functional predisposition to pancreatic beta cells. Thus, we used the highly sensitive and quantitative method, single cell digital PCR [21], to test whether genes important in beta cell function and development are expressed in neurogliaform cells of the neocortex. In particular, GLP1 receptors promote insulin secretion on pancreatic beta cells and expression of these receptors on insulin releasing neurons has been suggested to have therapeutic

implications [14]. We detected expression of GLP1 receptors in electrophysiologically and anatomically identified neurogliaform cells with single cell digital PCR (n = 11, Fig. 2a) using the homeostatic gene *S18* as reference. Moreover, we comparedcopy numbers of mRNAs of the *Glp1r* gene in neurogliaform cells (n = 5) in hypoglycaemia and found that hyperglycemic copy numbers exceeded that of hypoglycemic copy numbers by 9.6 times when normalized to copy numbers of the homeostatic *S18* gene (0.0457 ± 0.0427 and 0.0048 ± 0.0066, P<0.008, Mann Whitney U-test, Fig. 2a).

Next we asked whether GLP1 receptors and insulin can be co-detected in individualneurogliaform cells (Fig. 2b). Our single cell digital PCR method allows exact measurement of mRNA copy numbers of not more than two genes, thus we replaced the homeostatic gene *S18* with the *Ins2* gene in our protocol for testing GLP1 receptor and insulin co-expression. Similar to pancreatic beta cells, neurogliaform cells co-expressed mRNAs of the *Ins2* and *Glp1r* genes. Neurogliaform cells tested for co-expression in hyperglycemia (n = 5) contained higher numbers of mRNAs of both *Ins2*(8.60 ± 3.97) and *Glp1r*(8.40 ± 4.47) genes compared to neurogliaform cells (n = 5) in hypoglycaemia (2.60 ± 1.34 and 0.80 ± 1.30; P<0.037 and P<0.016, respectively, Mann Whitney U-test, Fig. 2b). These results on external glucose concentration modulated co-expression of insulin and GLP1 receptors in neurogliaform cells confirm our earlier report on insulin expression[13] and its glucose modulation in neurogliaform cells and corroborate the results shown above for *Glp1r* referenced to a homeostatic gene.

In order to confirm functional expression of GLP1 receptors, we tested the effect of GLP1 on electrophysiologically and anatomically identified neurogliaform cells using the hyperglycemic extracellular glucose concentration similar to earlier experiments [5](Fig. 2c-d). Measuring the current required for -90 mV holding potential in whole cell recordings before (-228 ± 39 pA, n = 11), during (-194 ± 49 pA, n = 11), and after (-214 ± 55 pA, n = 7), bath application of GLP1 (100 nM) [5], we detected a decrease in the holding current (P<0.003, Wilcoxon test) which was reversible upon washout (P<0.022, Fig. 2c-d). Moreover, pre-treatment with the GLP1 receptor specific antagonist exendin3(9-39) (1 µM) was effective in blocking the response in identified neurogliaform cells (n=7) to GLP1 application (-171 ± 39 pA vs. -166 ± 32 pA, P = 0.205, Wilcoxon test, Fig. 2e). Furthermore, changes in the holding current in neurogliaform cells (n=6) did not occur during application of GLP1 in hypoglycemic conditions (-201 ± 59 pA vs. -204 ± 58 pA, P = 0.401, Wilcoxon test, Fig. 2f). Accordingly, the moderate copy numbers relative to a homeostatic gene detected by single cell digital PCR in neurogliaform cells appear sufficient for a functional GLP1 response in neurogliaform interneurons.

The co-expression of GLP1 receptors and insulin in neurogliaform cells gives rise to a potentially broader molecular similarity between pancreatic beta cell as and neurogliaform neurons of the cerebral

cortex. Indeed, the developmental lineage for pancreatic endocrine cells and neurons has been suggestedto be related [23].Following these ideas, our final series of experiments using single cell digital PCR on identifiedneurogliaform cells revealed expression of transcription factors important in beta cell development [24, 25](*Pdx1, Isl1, Mafb*;copy numbers relative to *S18*, 0.0755 ± 0.0395, 0.0218 ± 0.0057, 0.0279 ± 0.0254, respectively Fig. 3). In addition, we detected significant increase in *Pdx1* and *Isl1* mRNA copy numbersin hyperglycemia (0.0073 ± 0.0163, 0.0051 ± 0.0072;$P<0.037$ and $P<0.016$, respectively, Mann Whitney U-test, Fig. 3).

Discussion

Our results provide evidence for GLP1 receptor expression in neurons known to release insulin in the cerebral cortex. Hyperglycemia increases the expression of GLP1 receptors in neurogliaform cells suggesting that endogenous incretins and therapeutic GLP1 receptor agonists might have effects on these neurons similar to that of pancreatic beta cells. In addition, we detected transcription factors (*Pdx1, Isl1, Mafb*) in neurogliaform cells known to be important in beta cell development.

The crucial gene in GLP1-insulin interaction, *Ins2,* shows significant variations when applying similar experimental conditions from cell type to cell type in the cerebral cortex [13], thus identification of the interneuron type(s) involved in the analysis is essential for appropriate interpretation of the results. Our combined electrophysiological, neuroanatomical and molecular techniques allowed us to monitor transcriptional changes associated with experimentally controlled alterations in extracellular glucose concentrations together with the ability to determine the identity of each neuron included in ourdataset. Previous analysis of transcriptional changes in identified neurons in response to variable glucose concentrations is scarce [13], but consistent regarding the functional effectiveness of ~10 copies of the *Ins2* and *Glp1r* genes per neuron. The expression threshold for functional GLP1 receptor response seems to be above 2 copies of *Glp1r* mRNAs in low external glucose concentrations. This suggests that genes with moderate expression levels detected by microarray or next generation techniques [26–30] and potentially interesting in insulin/incretin action are worth testing in functional experiments.

Conjoint modulation of the expression of the *Ins2* and *Glp1r* genes reported here in identified neurogliaform interneurons suggest that mechanisms classically described in the pancreas for GLP1-induced enhancement of insulin release might also operate in the brain. Application of glibenclamide known to promote insulin release from pancreatic beta cells has been successful in triggering insulin release from neurogliaform cells [13]. Although direct action of endogenous incretins or other GLP1

receptor agonists in neuronal insulin release requires further experiments, the mode of GLP1 action and the polarity of responses might be cell type specific [5]. We speculate that the outward current in response to GLP1 in neurogliaform cells at the holding potential applied in this study support that activation of GLP1 receptors lead to the opening of somatic K-channels possibly linked to GABAB receptors and, as suggested in response to GLP1 in hypothalamic neurons [31], to increased presynaptic GABA release. Moreover, considering the effect of GLP1 in the enhancement of synaptic and tonic inhibitory currents arriving to hippocampal pyramidal cells [32]and taking into account the high expression of extrasynaptic GABAA receptor delta subunits found on intermediate and distal dendrites of neurogliaform cells [33], we cannot exclude the possibility that our results also reflect the activation GABAA channels located on distal dendrites and detected with poor space-clamp due to the relatively low input resistance of neurogliaform cells[33, 34]. Possible tonic GABAA currents induced by GLP1 on neurogliaform cells are in line with the involvement of neurogliaform cells shown to provide synaptic and extrasynaptic inhibition [33] and it is further supported by insulin triggered tonic inhibition through GABAA receptors [35]. It is not yet clear whether neurogliaform cells receive innervation from GLP1 releasing neurons of the brainstem [5, 7, 8], however, in case intestinal-derived GLP1 or therapeutic GLP1 receptor agonists reach the cerebral cortex through the blood brain barriersimilar to native GLP1[3, 4] and could modulate insulin release from neurogliaform cells. The inhibition of gastric emptying is considered as apotentialfactor leading to weight loss caused by GLP1 receptor agonist therapy [2]. However, an alternative mechanism might emerge when considering imaging studies suggesting that the prefrontal cortex is important in the inhibitory control of food intake in humans [36–38]and human brain slice experiments showing that neurogliaform cells provide widespread inhibition in prefrontal microcircuits [39]. Selective involvement of GABAergic interneuron subpopulations is likely in neurodegenerative diseases[40]. Given that GLP-1 receptor agonists promise therapeutic effectiveness against neurodegeneration in models of traumatic brain injury and Alzheimer's and Parkinson's disease[16–20], the scenario of GLP-1 receptor mediated insulin synthesis in the brain could be extended to the therapy of these diseases.

A related developmental lineage for pancreatic endocrine cells and neurons has been implicated [23] and our results on the limited number of transcription factors tested in neurogliaform cells here support this idea. The pancreatic duodenal homeobox gene-1 (*Pdx1*) is central in the regulation of pancreatic development and in the differentiation of beta cells from progenitor cells[24]. The effects of GLP1 on beta cell proliferation and its secretory function depends on cross talk with proteins in the insulin signalling pathway and by modulation of transcription factors including PDX1[24], thus the co-expression of *Glp1r*, *Pdx1* and the TIDMS pathway found in neurogliaform cells suggest potential

functional homology of neurogliaform and beta cells beyond development. Along the same vein, expression of the LIM homeodomain protein ISL1 (insulin gene enhancer binding protein, islet factor 1) is known from the developing pancreas and the central nervous system [41, 42]. Synergistically with the basic helix-loop-helix transcription factor BETA2, ISL1 activates the insulin promoter in beta cells [43], promotes pancreatic islet cell proliferation [44] and ISL1 is required for the differentiation of interneurons in the spinal chord[45]. The role of musculoaponeuroticfibrosarcoma oncogene family A and B (*Mafa* and *Mafb*) genes is crucial in beta cells during development (*Mafb*) and adulthood (*Mafa* in mouse and *MAFA* and *MAFB* in human) [46] and our results in neurogliaform cells confirm the widespread expression of MAFB reported earlier in developing and differentiated neocortical interneurons [47]. Our results suggest that insulin and GLP1R expressing neurogliaform interneurons of the cerebral cortex partially possess the transcription toolkit known to be instrumental in the development of insulin synthesizing pancreatic beta cells.

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Duality of interest

The authors declare no conflict of interest.

Contribution statement

E.C. and G.T. formulated the key hypothesis, designed experiments, contributed to data analysis, visualization and interpretation and wrote the paper, Á.K.K., N.F.,E.C., Á.Z., I.L, A.P. and L.G.P. performed single cell digital PCR, molecular data interpretation and drafted the article, S.F., B.K., S.L.

and M.G. performed electrophysiology, harvested cytoplasms and drafted the text, performed analysis, visualization and drafted the manuscript.

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Figures

Fig. 1.

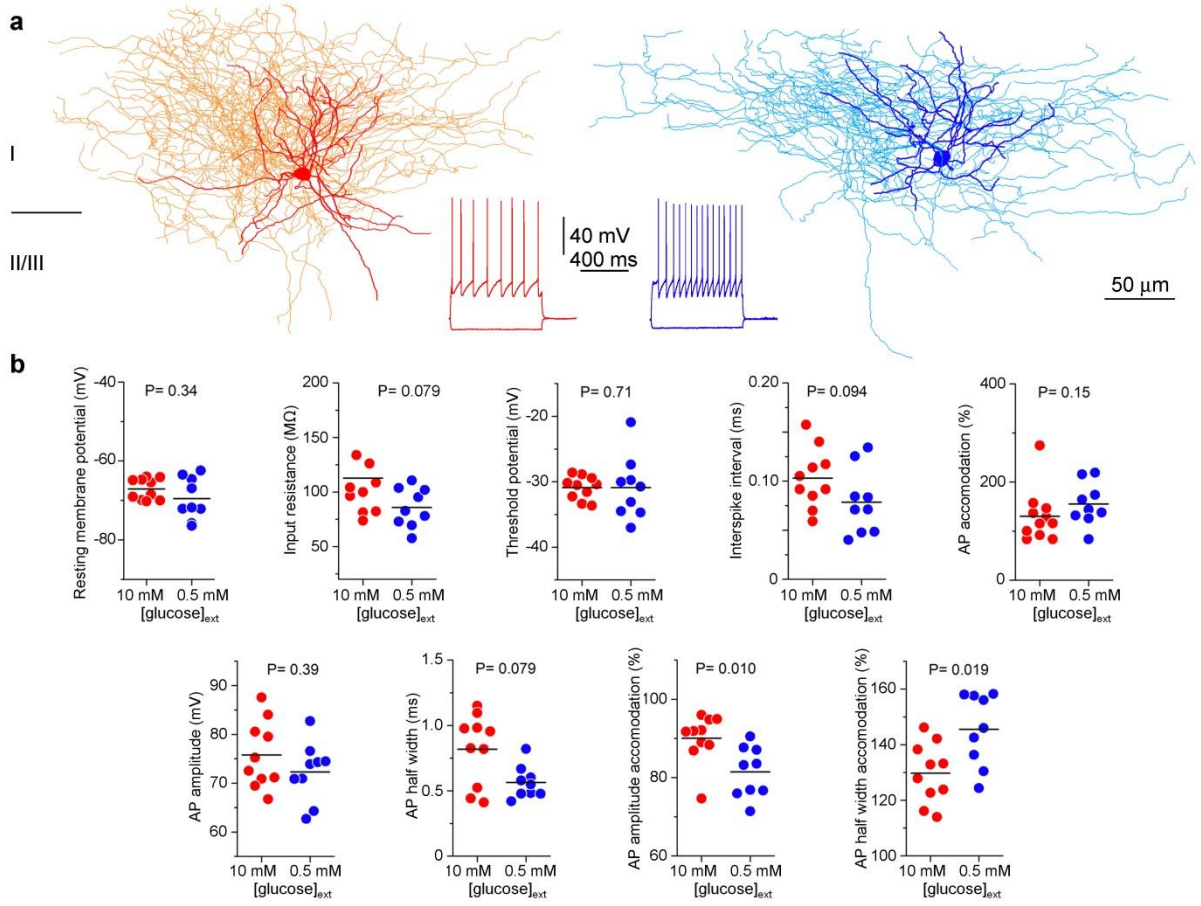


Fig. 2.

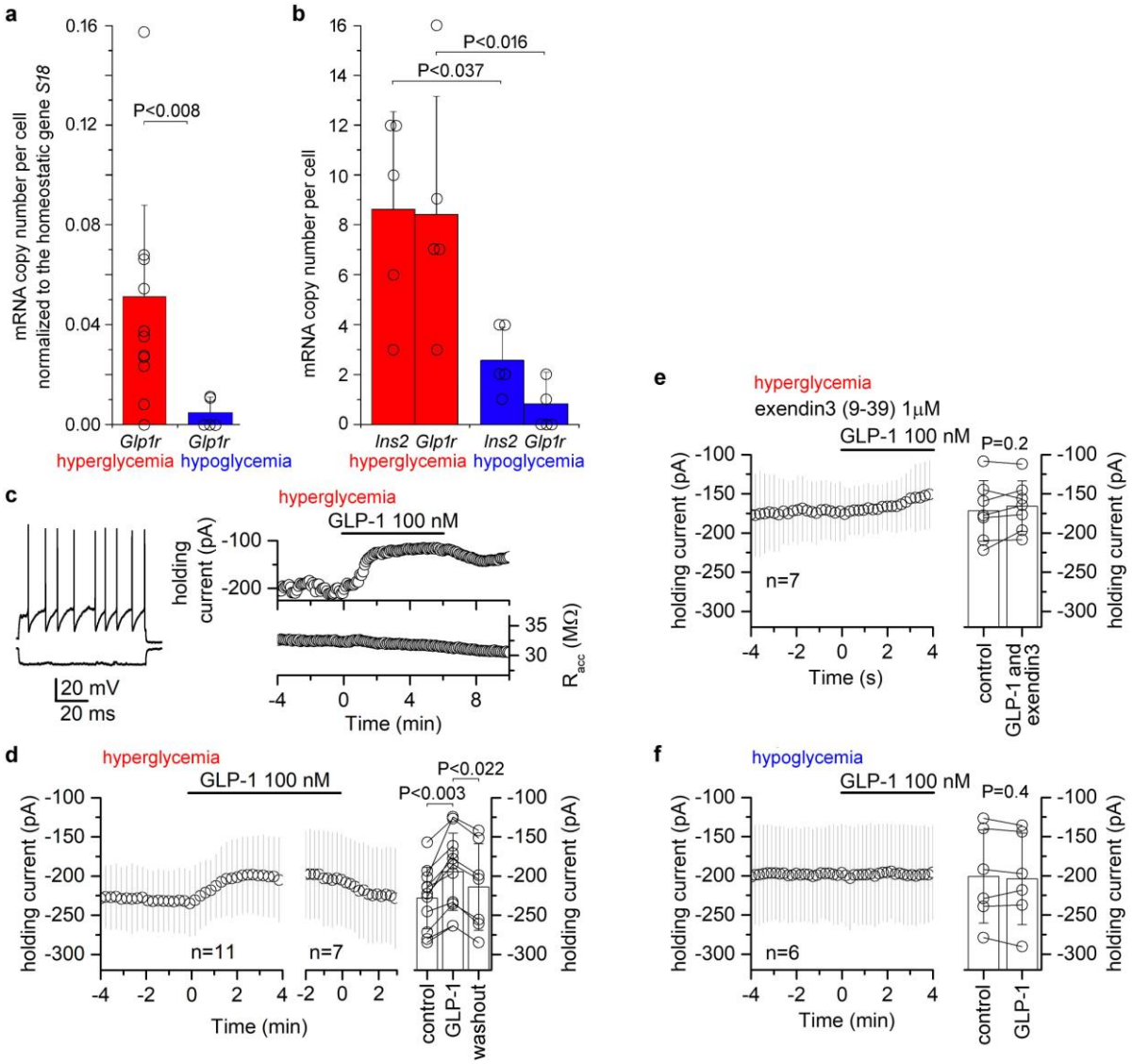
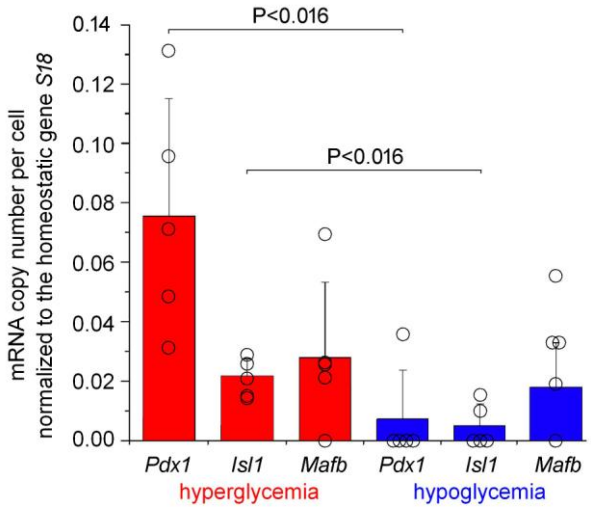

a
mRNA copy number per cell normalized to the homeostatic gene S18
P<0.008
Glp1r Glp1r
hyperglycemia hypoglycemia
b
mRNA copy number per cell
P<0.016
P<0.037
Ins2 Glp1r Ins2 Glp1r
hyperglycemia hypoglycemia
c
hyperglycemia
GLP-1 100 nM
holding current (pA)
20 mV
20 ms
Time (min)
R_acc (MΩ)
d
hyperglycemia
GLP-1 100 nM
P<0.022
P<0.003
n=11
n=7
Time (min)
control
GLP-1
washout
holding current (pA)
e
hyperglycemia
exendin3 (9-39) 1μM
GLP-1 100 nM
P=0.2
n=7
Time (s)
control
GLP-1 and exendin3
f
hypoglycemia
GLP-1 100 nM
P=0.4
n=6
Time (min)
control
GLP-1

Fig. 3.


P<0.016
P<0.016
mRNA copy number per cell
normalized to the homeostatic gene S18
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Pdx1
Isl1
Mafb
Pdx1
Isl1
Mafb
hyperglycemia
hypoglycemia

Legends for figures

Fig. 1

Anatomical and electrophysiological features of neurogliaform cells harvested for transcriptomic analysis. (**a**) Three dimensional reconstructions and firing patterns of neurogliaform cells whole cell patch clamp recorded and subsequently harvested for molecular analysis in brain slices of rat frontal cortex. The neurogliaform cell on the left (soma, dendrites and somatically recorded firing pattern, red; axon, orange) was recorded having hyperglycemic external glucose concentration (10 mM) in the artificial cerebrospinal fluid, which is standard in brain slice experiments. The neurogliaform cell on the right (soma, dendrites and somatically recorded firing pattern, blue; axon, light blue) was recorded in artificial cerebrospinal fluid containing 0.5 mM glucose reported during hypoglycaemia in the brain [22]. (**b**) Electrophysiological parameters of neurogliaform cell measured in hyperglicemia (red) and hypoglicemia (blue). Basic membrane parameters were not significantly different, however, the amplitude of action potentials (Aps) during a train decreased at a significantly higher rate in neurogliaform cells recorded in hypoglycaemia and the half width of their successive action potentials increased more rapidly compared to neurogliaform cell measured in hyperglycemia.

Fig. 2

Functional expression of GLP1 receptors in neurogliaform cells. (**a**) Expression of GLP1 receptors in electrophysiologically and anatomically identified neurogliaform cells detected by single cell digital PCR. Copy numbers of Glp1r mRNAs were higher in hyperglycemiacompared to hypoglycaemia when normalized to copy numbers of the homeostatic *S18* gene. (**b**) Co-expression of GLP1 receptors and insulin in individual neurogliaform cells. Neurogliaform cells tested for co-expression in hyperglycemia contained higher numbers of mRNAs of both Ins2 and Glp1r genes compared to neurogliaformcells in hypoglycaemia. (**c-f**) Functional expression of GLP1 on neurogliaform cells.(**c**) Example of an experiment testing the effect of GLP1 (100 nM) on identified neurogliaform cells. Left, Firing pattern of a neurogliaform cell recorded having hyperglycemic external glucose concentration (10 mM) in the artificial cerebrospinal fluid. Right, Application of 100 nM GLP1 to the neurogliaform cell (same as on left) alters the current required for -90 mV holding potential. (**d**) Population data confirm a decrease in the holding current which was reversible upon washout. Four out of 11 experiments were terminated before washout due to unstable access resistance.(**e**) Pre-treatment with the GLP1 receptor specific antagonist exendin3(9-39) (1 μM) blocked the response in identified neurogliaform cells to GLP1 application.(**f**) Changes in the holding current in neurogliaform cells did not occur during application of GLP1 in hypoglycaemia.

Fig. 3

Single cell digital PCR of neurogliaform cells confirms expression of transcription factors important in beta cells. Changes in extracellular glucose concentrations modulate the copy number of *Isl1* and *Pdx* mRNAs in electrophysiologically and anatomically identified neurogliaform cells.