Brief Communication

MOLECULAR METABOLISM

The glutamate receptor GluK2 contributes to the regulation of glucose homeostasis and its deterioration during aging

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ABSTRACT

Objective: Islets secrete neurotransmitters including glutamate which participate in fine regulation of islet function. The excitatory ionotropic glutamate receptor GluK2 of the kainate receptor family is widely expressed in brain and also found in islets, mainly in α and γ cells. α cells co-release glucagon and glutamate and the latter increases glucagon release via ionotropic glutamate receptors. However, neither the precise nature of the ionotropic glutamate receptor involved nor its role in glucose homeostasis is known. As isoform specific pharmacology is not available, we investigated this question in constitutive GluK2 knock-out mice (GluK2$^{-/-}$) using adult and middle-aged animals to also gain insight in a potential role during aging.

Methods: We compared wild-type GluK2$^{+/+}$ and knock-out GluK2$^{-/-}$ mice using adult (14—20 weeks) and middle-aged animals (40—52 weeks). Glucose (oral OGTT and intraperitoneal IPGTT) and insulin tolerance as well as pyruvate challenge tests were performed according to standard procedures. Parasympathetic activity, which stimulates hormones secretion, was measured by electrophysiology in vivo. Isolated islets were used in vitro to determine islet β-cell electrical activity on multi-electrode arrays and dynamic secretion of insulin as well as glucagon was determined by ELISA.

Results: Adult GluK2$^{-/-}$ mice exhibit an improved glucose tolerance (OGTT and IPGTT), and this was also apparent in middle-aged mice, whereas the outcome of pyruvate challenge was slightly improved only in middle-aged GluK2$^{-/-}$ mice. Similarly, insulin sensitivity was markedly enhanced in middle-aged GluK2$^{-/-}$ animals. Basal and glucose-induced insulin secretion in vivo was slightly lower in GluK2$^{-/-}$ mice, whereas fasting glucagonemia was strongly reduced. In vivo recordings of parasympathetic activity showed an increase in basal activity in GluK2$^{-/-}$ mice which represents most likely an adaptive mechanism to counteract hypoglucagonemia rather than altered neuronal mechanism. In vitro recording demonstrated an improvement of glucose-induced electrical activity of β-cells in islets obtained from GluK2$^{-/-}$ mice at both ages. Finally, glucose-induced insulin secretion in vitro was increased in GluK2$^{-/-}$ islets, whereas glucagon secretion at 2 mmol/l of glucose was considerably reduced.

Conclusions: These observations indicate a general role for kainate receptors in glucose homeostasis and specifically suggest a negative effect of GluK2 on glucose homeostasis and preservation of islet function during aging. Our observations raise the possibility that blockade of GluK2 may provide benefits in glucose homeostasis especially during aging.

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Keywords Islets; Kainate receptor; *GRIK2*; GluK2; Aging; Microelectrode array

1. INTRODUCTION

The hormone secreting pancreatic islets are central to the maintenance of glucose homeostasis. Their activity and interaction is finely tuned by a number of autocrine and paracrine factors, which enhance coordination among the different hormone secreting cell-types present in islets, i.e. insulin-containing β-cells, glucagon-containing α-cells, somatostatin secreting δ-cells, pancreatic polypeptide secreting γ-cells as well as a small number of ghrelin secreting ϵ-cells [1,2]. One such factor, the amino acid glutamate, which acts as an excitatory

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Abbreviations: GluK2, kainate receptor subunit GluK2; MEA, microelectrode array; OGTT, oral glucose tolerance test; IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; PyrCT, pyruvate challenge test

neurotransmitter in the brain, is present in exocytotic peptide-hormone containing large dense core vesicles as well as synaptic-like microvesicles in α- and β-cells [3—5]. Quantitatively, α-cells seem to be the most important source of regulated glutamate release [4—6] although glutamate derived from metabolic pools via reversal of uptake by membrane glutamate transporters has been proposed to contribute [7].

Glutamate activates metabotropic receptors, which are coupled to G-proteins and downstream pathways, or ionotropic receptors, such as the NMDA-, AMPA- and kainate receptors forming non-selective cation channels [8]. Isoforms of most of these ionotropic receptors are expressed to various degrees in islet cells, though at lower levels than in brain [9]. No major difference exists in mouse as compared to man concerning the relative distribution between α- and β-cells [10]. The ionotropic NMDA receptors (GluN1 to 3, gene symbol *GRIN1* to *3* [11]) are ligand-gated channels permeable to calcium, and their activity is highly dependent on voltage [12]. The islet NMDA receptor GluN1 plays an inhibitory role in glucose-induced insulin secretion and has been characterized as drug target in diabetes therapy [13—15]. As membrane depolarisation plays a role and high-affinity binding of glutamate as well as co-activation by the amino acids glycine or serine are required for NMDA channel opening, these receptors may be saturated by the prevailing extracellular plasma concentrations of the corresponding amino acids [16]. In contrast, ionotropic AMPA receptors (GluA1 to 4, gene symbol *GRIA1* to 4 [11,17]) and kainate receptors (GluK1 to 5, gene symbol *GRIK1* to 5 [11,17]) are physiologically stimulated only by glutamate and require higher concentrations of glutamate similar to those seen locally after release of this amino acid from islet cells [4,12].

Glutamate activation of islet AMPA and kainate receptors stimulates secretory cells in an auto- or paracrine manner [4,18—22]. Final outcome, however, may be more complex; for example, activation of a variant of the AMPA receptor GluA4 on δ-cells leads to secretion of somatostatin [20], which in turn inhibits α- and β-cells [23,24]. Similarly, activation of α-cell glutamate receptors and ensuing glucagon release may enhance insulin secretion from β-cells [25].

Subsequent to the initial observation in rodents that activation of α-cells leads to glutamate release [4], a series of elegant experiments led to the demonstration of a positive regulatory loop of glucagon release via co-secreted glutamate through AMPA and/or kainate receptors in primate islets and its acute functional relevance in vivo in mice [18]. The precise receptor type(s) involved, however, remained unclear, and pharmacology did not offer specific tools to determine what they were [11]. At least four of the five kainate receptor subunits are expressed at the RNA level in murine and human islets, i.e. GluK2 to GluK5 [10]. In contrast to GluK2 and GluK5, GluK1, GluK3, and GluK4 are present only at very low or spurious levels in murine or human islets [10,26]. Moreover, GluK2 and 3 can function as homomers, whereas GluK4 and 5 must assemble as obligatory heterotetramers with GluK1, 2, or 3 [27—29]. Therefore GluK2 may be a promising target to investigate the potential role of kainate receptors in islet physiology as its inactivation also impedes GluK5 protein expression [30]. Within islets GluK2 is found mainly on α-cells and on pancreatic polypeptide producing γ-cells [10,26]. Outside islets GluK2 is widely expressed in brain tissue and is implicated in the fine regulation of the activity of neuronal circuits by acting at the pre- or postsynaptic side, through either ionotropic or metabotropic functions [28]. It has also been implicated in the propensity to recurrent seizures in chronic epilepsy [28,31]. Except for a lesser increase in body weight and despite reduced motor activity, no changes of potentially metabolic origin have been described in GluK2 knock-out mice [31—34].

In view of the lack in specificity of drugs, we took advantage of an existing, constitutive knock-out model [25,32,36]. As the mechanisms involved are expected to concern fine tuning of islet function, but not basic mechanisms in the control of glucose homeostasis, we hypothesized that changes may become more prominent during aging and observations in middle-aged mice, in contrast to old mice, may avoid interference by a general senescence phenotype [37]. For this reason we investigated both adult and middle-aged mice to test whether GluK2 may play a role in islet function and glucose homeostasis.

2. MATERIALS AND METHODS

2.1. Animals and cells
Progeny of the constitutive knock-out C57BL/6 GluK2$^{-/-}$ [32,35,36] and wild-type C57BL/6 mice were bred at the Bordeaux University Animal House Facility (DAP 04236.01, DAP 15318). C57BL/6J GluK2$^{-/-}$ had been backcrossed 10 times with C57BL/6J and subsequently wild-type C57BL/6J GluK2$^{+/+}$ mice and C57BL/6J GluK2$^{-/-}$ mutant litter mice were obtained by crossing heterozygous mice. Animals were genotyped by RT-PCR (primers see Supplemental Table 1) using a KAPA Mouse Genotyping Kit (KAPA Biosystems, Boston, MA, USA). Animals (male) were used at adult (14—20 weeks) or at middle-aged age (40—52 weeks). All experimental procedures were approved by the Ministry of Education and Research (no. 04236.01).

2.2. Glucose and insulin tolerance tests, pyruvate challenge, islet secretion assays, and morphometric analysis
Glucose (OGTT, 3 g/kg; IPGTT 2 g/kg) and insulin tolerance tests (0.5 U/kg) were performed as described [38,39]. Similarly, glycemia was measured during an intraperitoneal pyruvate challenge (2 g of pyruvate/kg body weight) after overnight fasting (15 h). Islets were obtained as published [39,40] and cultured overnight in RPMI (containing 11 mM glucose and supplemented with FCS 10%, penistroptomycin 1%, glutamine 1%, HEPES 10 mM, pyruvate 1 mM) at 37 °C (5% CO_2, >90% relative humidity) in 96-well filter plates (multiscreen Durapore BV1.2 μm; Millipore, Molsheim, France). Dynamic insulin and glucagon release was assayed as published [41]. Blood glucose concentrations were determined with ACCU-CHEK (Roche). Pancreatic mass and α/β cell mass were measured on isolated whole pancreas as described previously [42] and further detailed in the legend to Figure S1.

2.3. In vivo recordings of parasympathetic activity
Parasympathetic activity was recorded at the thoracic branch of the vagus nerve, along the carotid artery as described previously [43]. Unipolar nerve activity was recorded continuously for 15 min during fasting state and after an intraperitoneal injection of glucose (2 mg/kg) during 15 min. Data were digitized with PowerLab/4sp digitizer (ADinstruments, Paris, France). Signals were amplified 105 times and filtered using low- and high-frequency cut-offs of 100 and 1000 Hz and monitored using the Chart 4 computer program (ADinstruments, Paris, France).

2.4. Islet isolation, cell culture, electrophysiology and signal analysis, immunofluorescence
Electrophysiological recordings were performed according to published methods [44—46]. Mice islets were prepared and seeded on microelectrode arrays (MEA, 60 electrodes; MCS, Reuttlingen, Germany). Four days later, signals were recorded (10 kHz) and analyzed offline in terms of frequency with commercial software (MC_Rack, MCS). Immunofluorescence was performed on whole islets and dispersed
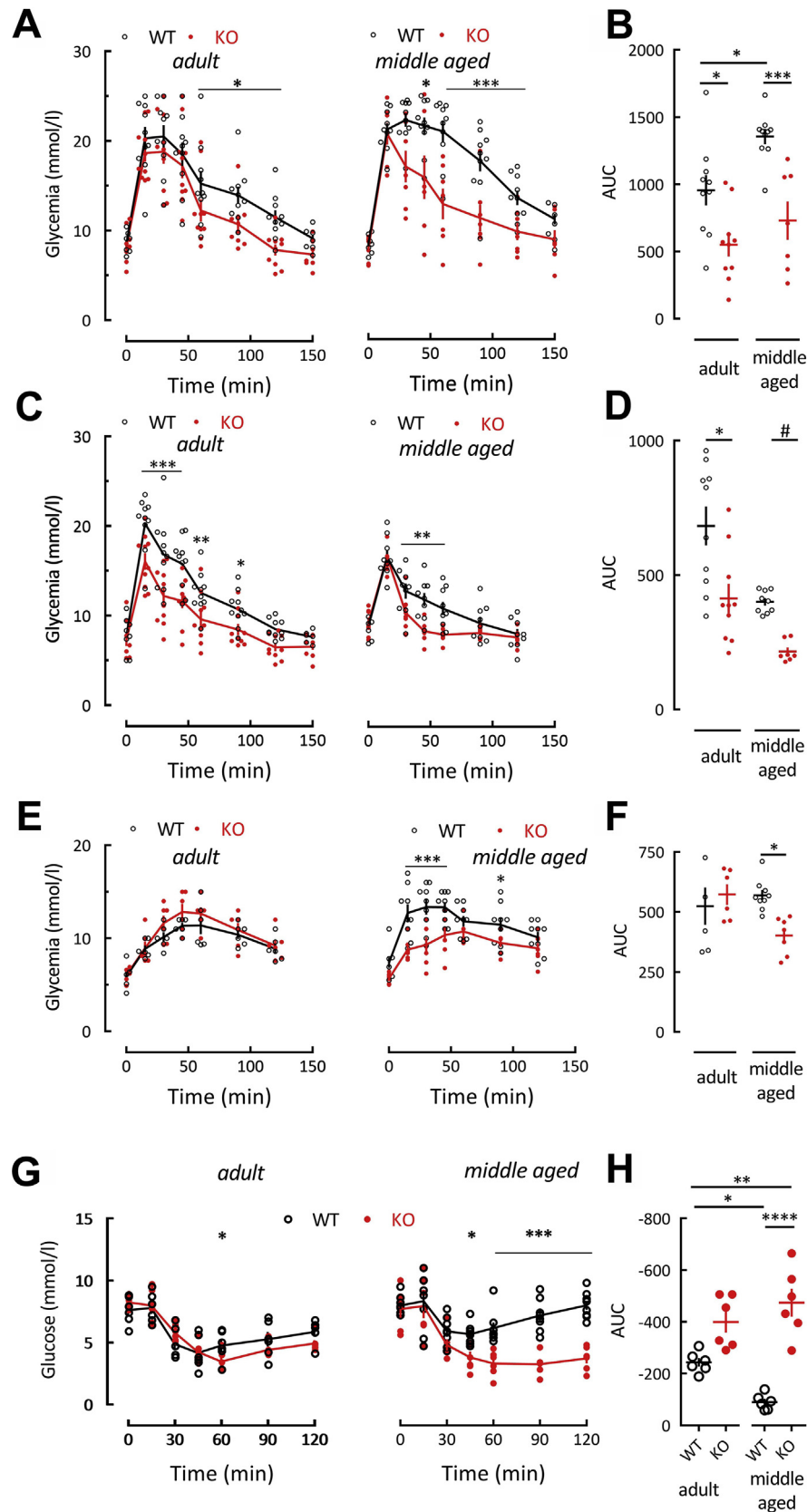
Figure 1: Improved glucose tolerance, pyruvate challenge outcome and insulin sensitivity in GluK2$^{-/-}$ mice. Given are time courses and the area under the curve (AUC). (**a, b**) Intraperitoneal Glucose Tolerance Test (**c, d**), Oral Glucose Tolerance Test (OGTT) and (**e,f**) Intraperitoneal Pyruvate Challenge Test were performed in WT and KO mice, in both adult and middle-aged animals. (**g**) Insulin Tolerance Test (0.5 U/kg) was performed in WT (open circles) and KO mice (closed red circles), both in adults and middle-aged. (**h**) Area under the curves of g (AUC). Means ± SEM; a, c, e and g, t-test; b, d, f and h, ANOVA followed by Tukey post-hoc test; *p < 0.05, **p < 0.01, ***p < 0.001 or by Holm-Sidak post-hoc test, #p < 0.05; N = 7 except for g and h (N = 6).

islet cells using either a polyclonal rabbit anti-GluK2 antibody (Synaptic Systems, Goettingen, Germany) [47] or a rabbit monoclonal antibody (anti-GluR6/7 clone NL9, Merck Millipore) [48] and appropriate secondary antibodies.

2.5. Statistics

Values are expressed as mean $\pm$ SEM. D'Agostino-Pearson omnibus or Brown–Forsythe tests were used to test for normality. Comparison of means was made by tests as indicated in the figure legends using Prism 7.0 and AUC's were calculated as values above basal using the corresponding function in the same software.

3. RESULTS

3.1. GluK2 knock-out improves glucose tolerance and insulin sensitivity

To obtain a first indication whether the constitutive knock-out of excitatory GluK2-containing kainate receptors alters glucose homeostasis, we tested glucose tolerance [49] (Figure 1). Intraperitoneal glucose tolerance tests (IPGTTs) showed a significant decrease in glucose-tolerance in middle-aged GluK2$^{+/+}$ mice as compared to adult ones (Figure 1A,B). In contrast, an improved tolerance was evident in adult GluK2$^{-/-}$ mice when compared to GluK2$^{+/+}$ animals at time points later than 50 min. This improved glucose handling was preserved in middle-aged GluK2$^{-/-}$ mice (Figure 1A,B). Oral glucose tolerance tests are more directly influenced by islet function. Using this test GluK2$^{-/-}$ mice showed again an improved tolerance, which was somewhat less marked than in OGTT but well preserved during aging (Figure 1C,D). We also examined pyruvate challenge, which reflects gluconeogenesis in the liver and in other organs [49]. Whereas no difference was evident between adult wild-type or knock-out mice, however, a clear improvement in pyruvate challenge tests was apparent in middle-aged GluK2$^{-/-}$ mice as compared to middle-aged GluK2$^{+/+}$ animals (Figure 1E,F). Insulin sensitivity was comparable among adult GluK2$^{+/+}$ or GluK2$^{-/-}$ mice and, as expected, sensitivity deteriorated significantly during aging in wild-type animals (Figure 1G,H). In stark contrast, in middle-aged GluK2$^{-/-}$ mice insulin sensitivity was well preserved, similar to adult GluK2$^{-/-}$ animals and even slightly better than in adult wild-type mice. In view of the above described phenotype, we concentrated our investigations on middle-aged animals. Note that pancreas weight as well as α- or β-cell volume did not differ among wild-type and knock-out mice (Fig. S1). As previously reported [32], we observed a smaller weight gain in GluK2$^{-/-}$ mice (Fig. S2). Note that we were not able to detect GluK2 by immunofluorescence in islets in line with their low expression levels in these tissues (data not shown). Collectively, these data suggest a remarkable preservation of glucose handling in GluK2$^{-/-}$ mice during aging.

3.2. Knockout of GluK2 reduces fasting glucagonemia and increases parasympathetic nerve activity

We next tested insulin and glucagon blood levels either after a glucose challenge or at the fasting state (Figure 2). Basal insulin levels were lower in GluK2$^{-/-}$ mice in vivo as well as 30 min after intraperitoneal glucose injection in line with the improved glucose handling and insulin sensitivity observed in Figure 1. Interestingly we also observed a significant reduction in fasting glucagon levels in GluK2$^{-/-}$ mice as compared to wild-type animals.

In vivo islet function is influenced by the central nervous system via the parasympathetic vagus nerve, which stimulates insulin and glucagon secretion [50], and its efferent branch is activated upon an
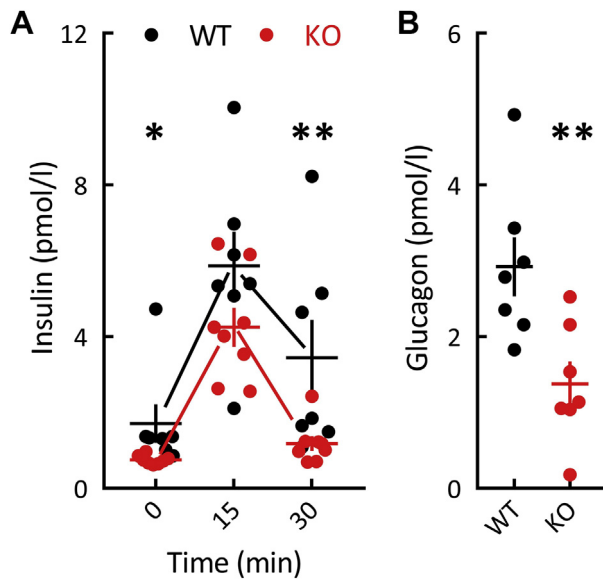

Figure 2: Middle-aged GluK2$^{-/-}$ mice have reduced basal and glucose-stimulated insulinemia as wells reduced fasting glucagonemia. (a) Insulin levels in vivo were lower in KO mice. Means $\pm$ SEM; N = 7. (b) Fasting glucagonemia is significantly reduced in middle-aged KO mice. Means $\pm$ SEM; N = 7. Mann–Whitney; * 2p < 0.05, ** 2p < 0.01.

increase in glucose [51,52]. We therefore examined whether the decrease in plasma glucagon levels may be due to a reduced parasympathetic activity in knock-out animals. Recordings of electrical activity in fasted wild-type mice showed a clear increase in nerve activity when glucose levels were increased via a glucose injection (Figure 3). Contrary to our expectations, in GluK2$^{-/-}$ mice, electrical activity was already significantly elevated in the fasted state and glucose injections did only led to a minor additional increase (Figure 3).

3.3. Knockout of GluK2 preserves electrical in vitro activity of islets during aging

The in vivo phenotyping presented above begs the question whether in vitro islet function may be altered in GluK2$^{-/-}$ mice. We first examined electrical activity of islets cells by extracellular recordings using microelectrode arrays. Under the conditions used the recording of so-called slow potentials reflects biphasic activity of β-cells and depends strictly on glucose concentrations applied [40,46,53—55]. Note that this approach does not capture electrical activity of α-cells as most likely they are not coupled and henceforth electrical signals are too small. As can be seen in Figure 4A,C, an increase in glucose from 3 to 8.2 mM induces a vigorous electrical activity in islets prepared from adult wild-type GluK2$^{+/+}$ mice. Note that a distinct first phase is not always clearly visible here as means are given and activation may not be completely in phase in between the different islets on the same micro-electrode array. Islets from adult GluK2$^{-/-}$ mice had a significantly higher level of activity (Figure 4A,C). This is evident during the first and even more pronounced during the second phase of glucose stimulation. Most importantly this difference between GluK2$^{+/+}$ and GluK2$^{-/-}$ mice was fully conserved during aging (Figure 4B,D) and suggests that disruption of the gene encoding GluK2 improved glucose-induced electrical coupling of β-cells during the first and the second phase of islet activity.

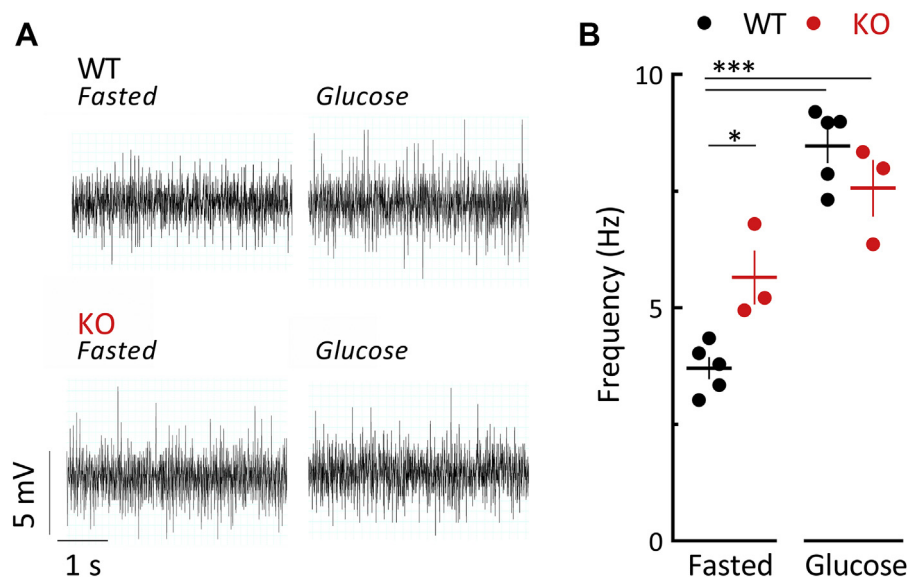**Figure 3:** Middle-aged GluK2$^{-/-}$ mice have an increased electrical activity of the parasympathetic nerve in the fasted state. (a) Electrical signals of in vivo recordings during fasting state and after an intraperitoneal injection of glucose (2 mg/kg) in middle-aged GluK2$^{+/+}$ (WT) or GluK2$^{-/-}$ (KO) mice. (b) Statistics. Means ± SEM; ANOVA and Tukey's post-hoc analysis; *2p < 0.05, ***p < 0.001; N = 3−5.

3.4. Knockout of GluK2 improves glucose-induced insulin secretion but reduces glucagon secretion in vitro

We finally tested whether this improved activity is also reflected in islet hormone secretion. To this end, islets from middle-aged animals (GluK2$^{+/+}$ or GluK2$^{-/-}$) were superfused with 11 mM, 2 mM, and again 11 mM glucose (Figure 4E−F). Note that cell mass and hormone contents were not significantly different between GluK2$^{+/+}$ or GluK2$^{-/-}$ islets (see Supplemental Figs. 1 and 3). Islets obtained from GluK2$^{-/-}$ mice secreted significantly more insulin during the initial stimulation as well as during the second stimulus (Figure 4F,G). Note that this observation is not in contradiction to the data obtained in vivo where insulin secretion is also determined by insulin sensitivity. In contrast, glucagon secretion at 2 mM glucose was largely reduced in GluK2$^{-/-}$ (Figure 4H,K). Glucagon secretion was distinctive biphasic under the conditions used here, a phenomenon often observed when stimulations by arginine are used but also described previously for glucose in perfused pancreas [56,57] and in islets [58,59].

4. DISCUSSION

Glucose homeostasis is fine-tuned by neurotransmitter actions in the nervous system and locally within the pancreatic islets [1,60]. Major findings in our study on the knock-out of the excitatory kainate receptor subunit GluK2 were an improved glucose tolerance characterized by reduced glucagon release, increased insulin sensitivity, and enhanced insulin secretion in vitro as well as absence of the decline in these functions normally encountered during aging. The normal aspect of islets in terms of mass and α/β-cell volumes suggests the absence of any major developmental abnormality. Collectively, these data suggest that GluK2, which finely tunes the activity of neuronal circuits [28] and which has previously been implicated in recurrent epileptic seizures [31,34], autism [61], and cognitive abilities [62], exerts a wider biological role such as in glucose homeostasis.

Obviously, a major limitation of our study is the use of a constitutive knock-out and we can thus not pinpoint precisely the anatomical relevant site or sites. Indeed, the homeostatic effect observed here may be mediated by a peripheral action within the islet as well as a central action on neural regulation. Glutamatergic neurotransmission intervenes in regulation of glucose homeostasis, mainly investigated in the context of counterregulatory responses to hypoglycaemia [63], but the precise structures and connections are still far from being elucidated. In this context glutamate plays a role in ventromedial hypothalamic neurons [64] and via kainate receptors in the dorsal motor nucleus of the vagus that regulates stimulatory parasympathetic output to the islets [65]. Thus the increased electrical activity of the vagus observed here may reflect an altered excitatory output to islet α- and β-cells in the GluK2$^{-/-}$ mice. However, increased vagal activity should enhance islet hormone secretion in vivo but was accompanied here by reduced glucagon levels in vivo. Thus, the increased vagus activity more likely reflects a counterregulatory adaptation to the reduced α-cell activity, which we observed in vivo and in vitro in isolated islets.

The improved glucose tolerance clearly has several components. Note that differences in locomotor activity may obviously confound the metabolic situation. Although such a reduction has been described by one group [66], it was not found in our colony [33]. A difference in weight between wild type and knockout mice was observed here and in an earlier study [32] throughout all ages and is expected to improve glucose tolerance. However, insulin sensitivity was comparable in adult mice, thus at a stage where improved electrical β-cell activity was already present in vitro. This points at least to a sizeable contribution of islet mechanisms to the phenotype.

Different intra-islet glutamate effects on glucagon secretion have been described. At low glucose, glutamate, AMPA, or kainate stimulates α-cell glucagon secretion but not insulin release [18,19,22,25]. Note that one study reported an inhibitory effect of glutamate on glucagon secretion in rat islets via a metabotropic glutamate receptor (mGluR4, gene symbol GRM4) [67]. Similarly, glutamate may activate δ-cells [20] and the ensuing release of somatostatin may reduce α-cell activity, but this should occur at elevated levels of glucose [23].
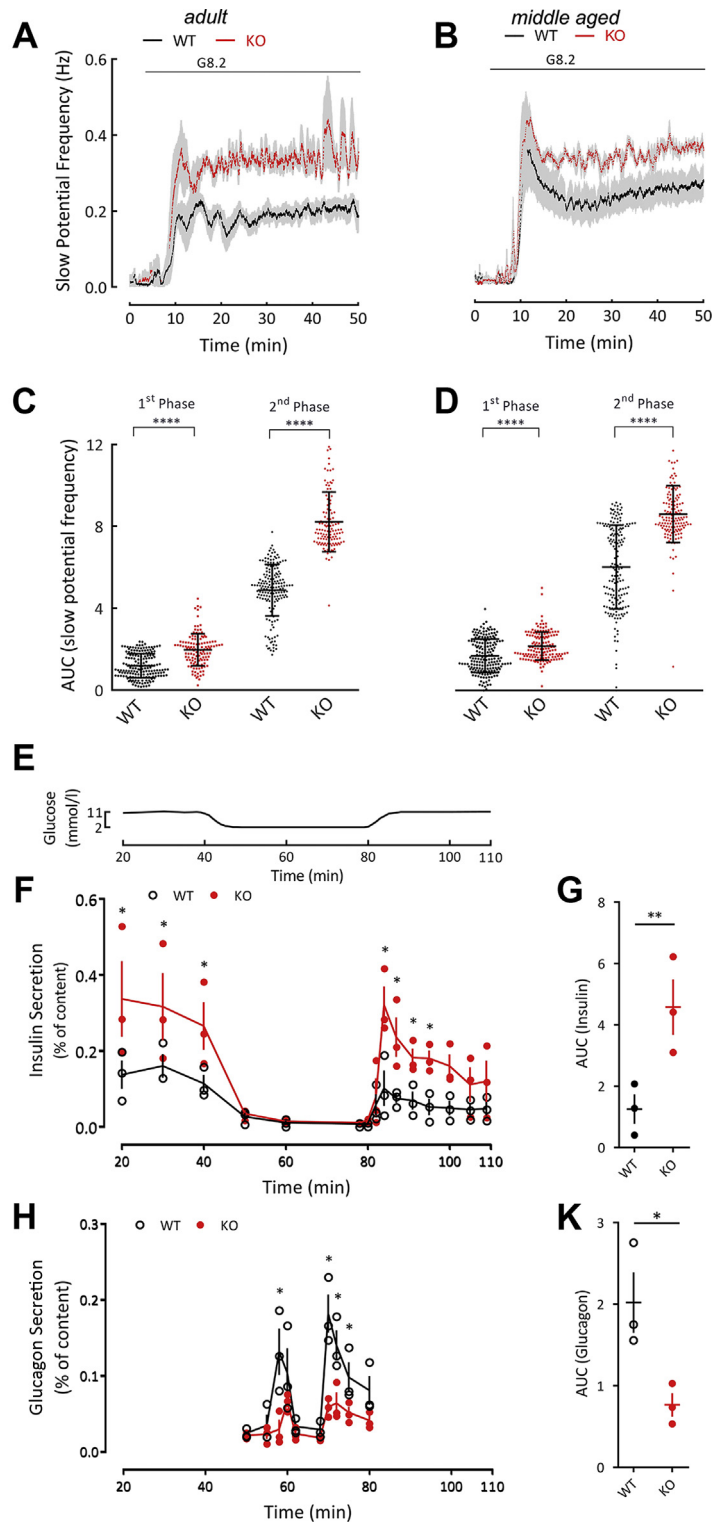
Figure 4: Increased glucose-induced islet β-cell activity in adult and middle aged GluK2$^{-/-}$ mice. (a−c) Electrical activity of islets was recorded in vitro on microelectrode arrays at 3 and 8.2 mM glucose. (a, b) Given are means and SEM of average slow potential frequency per mice (GluK2$^{+/+}$, WT; GluK2$^{-/-}$, KO; N = 4 independent preparations of mouse islets for each condition in adult (a) or middle aged (b) mice). (c, d) Areas under the curve (AUC) for first phase (5−15 min) and second phase (25−50 min). Given are means ± SD, N = 4 independent islet preparations and assays representing analysis of 126−183 islets for each condition (WT and KO, adult (c) or middle aged (d) mice). Mann−Whitney test; ****, 2p < 0.0001. (e) Glucose perfusion pattern (as used in f and h); initial perfusion at 11 mM glucose was reduced to 2 mM and subsequently raised again to 11 mM. (f) Insulin release (as glucose induced insulin secretion in regard to the value at 3 mM glucose at 60 min) for middle aged GluK2$^{+/+}$ (WT) and GluK2$^{-/-}$ (KO) mice. (g) Area under the curves for insulin (AUC, t = 82−110 min). Means ± SEM. (h) Glucagon release (as fold stimulation in regard to value at 11 mM glucose at 50 min) for middle-aged GluK2$^{+/+}$ (WT) and GluK2$^{-/-}$ (KO) mice. (k) Area under the curves for glucagon (AUC, t = 55−80 min). (f−k) Means ± SEM; N = 3 independent islet preparations and assays; t-test; * 2p < 0.05; ** 2p < 0.01. Hormone contents did not differ significantly between islets from WT and KO animals (see Supplemental Fig. S3).

In contrast, there is considerable evidence for a stimulatory autocrine feedback loop of glutamate in α-cells [4,18,20]. The obvious question is whether GluK2 may be specifically implicated. As α-cells express AMPA as well as kainate receptors, and both are difficult to distinguish in pharmacological terms, the molecular identity of the relevant receptors has hitherto only been addressed indirectly. Although previous reports favor a functional role of AMPA rather than of kainate receptors [19], caution should be exerted. Isolated α-cells may behave differently from cells within islets, as actually observed by the authors [19], and these findings do not preclude a role of kainate receptors specifically in the autocrine stimulation of glucagon secretion. The situation is even more complex in view of the reported metabotropic action of kainate receptors which may imply GluK2 [68—70]. Such a metabotropic action may not be detected in electrophysiological recordings. Finally it is also of note that antagonist used in this [19] as well as in other studies [22,25] are only partially specific at best [11].

GluK2 mutations have been implicated previously in neuronal diseases [61,71,72] but not in glucose homeostasis. Hints for a putative link to diabetes may be given by reports of strongly decreased retinal GluK2 expression in streptozotocin-induced diabetes in rats [73] and GWAS based association between a polymorphism near the GluK2 gene *GRIK2* and diabetic retinopathy in man [74]. Interestingly, several features of the phenotype observed here in GluK2$^{-/-}$ mice resembled to some extent experiments interfering with glucagon receptor activity or α-cell function [75—78]. The underlying scenario would be given by the lower levels of the hyperglycemic hormone glucagon and ensuing lesser needs of insulin in line with the lower levels of insulin during GTT and fasting glucagon which we observed here. In the long run, this would also lead to improved insulin sensitivity as measured here. The conservation of insulin sensitivity in middle-aged mice provides also the probable cause of the improved outcome in the pyruvate challenge tests in those animals as the latter was not altered in adult animals when insulin sensitivity was still comparable between Gluk2$^{+/+}$ and Gluk2$^{-/-}$ mice.

The improvement in islet function in terms of electrical activity and insulin secretion represents most likely an adaptive mechanism to the overall metabolic situation: a decreased demand on β-cell activity due to lower basal glucagon levels and, in middle-aged animals, additionally due to a conserved insulin sensitivity. Our data also suggest that these adaptive phenomena appear rather early, prior to differences in insulin sensitivity, as we observed increased glucose-induced electrical β-cell activity in vitro already in adult GluK2$^{-/-}$ mice. A direct effect on β-cells of lower α-cell derived glutamate or glucagon levels is rather unlikely. As both, glutamate and glucagon, stimulate insulin secretion in vitro [25,79], their reduction should lead to lower insulin secretion whereas an enhancement was observed here.

5. CONCLUSION

Collectively, our observations suggest that kainate receptors are implicated in glucose homeostasis, although the main initial mechanism may imply several sites. Moreover, our data suggest that the knockout of GluK2 may avoid the reduction in glucose homeostasis that is known to occur during aging [80]. This obviously comes at the cost of an increased risk of hypoglycemia, as evidenced here by the marked lowering in glucose levels during the insulin sensitivity test in GluK2$^{-/-}$ middle-aged animals. The glutamate/glucagon feedback loop may have provided an evolutionary advantage when food was scarce, and its blockade may be of interest in the modern situation often characterized by hypercaloric food supply. In this context, a detailed profiling of novel kainate-receptor antagonists [81] on glucose homeostasis and α-cell function could be of interest.

CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2019.09.011.

REFERENCES

[1] Caicedo, A., 2013. Paracrine and autocrine interactions in the human islet: more than meets the eye. Seminars in Cell and Developmental Biology 24: 11—21.

[2] Da Silva Xavier, G., 2018. The cells of the islets of Langerhans. Journal of Clinical Medicine 7.

[3] Hayashi, M., Morimoto, R., Yamamoto, A., Moriyama, Y., 2003. Expression and localization of vesicular glutamate transporters in pancreatic islets, upper gastrointestinal tract, and testis. Journal of Histochemistry and Cytochemistry 51:1375—1390.

[4] Hayashi, M., Yamada, H., Uehara, S., Morimoto, R., Muroyama, A., Yatsushiro, S., et al., 2003. Secretory granule-mediated co-secretion of L-glutamate and glucagon triggers glutamatergic signal transmission in islets of Langerhans. Journal of Biological Chemistry 278:1966—1974.

[5] Gammelsaeter, R., Coppola, T., Marcaggi, P., Storm-Mathisen, J., Chaudhry, F.A., Attwell, D., et al., 2011. A role for glutamate transporters in the regulation of insulin secretion. PLoS One 6:e22960.

[6] Weaver, C.D., Yao, T.L., Powers, A.C., Verdoorn, T.A., 1996. Differential expression of glutamate receptor subtypes in rat pancreatic islets. Journal of Biological Chemistry 271:12977—12984.

[7] Feldmann, N., del Rio, R.M., Gjinovci, A., Tamarit-Rodriguez, J., Wollheim, C.B., Wiederkehr, A., 2011. Reduction of plasma membrane glutamate transport potentiates insulin but not glucagon secretion in pancreatic islet cells. Molecular and Cellular Endocrinology 338:46—57.

[8] Willard, S.S., Koochekpour, S., 2013. Glutamate, glutamate receptors, and downstream signaling pathways. International Journal of Biological Sciences 9: 948—959.

[9] Otter, S., Lammert, E., 2016. Exciting times for pancreatic islets: glutamate signaling in endocrine cells. Trends in Endocrinology and Metabolism 27:177—188.

[10] Benner, C., van der Meulen, T., Caceres, E., Tigyi, K., Donaldson, C.J., Huising, M.O., 2014. The transcriptional landscape of mouse beta cells compared to human beta cells reveals notable species differences in long noncoding RNA and protein-coding gene expression. BMC Genomics 15:620.

[11] Alexander, S.P., Peters, J.A., Kelly, E., Marrion, N.V., Faccenda, E., Harding, S.D., et al., 2017. The concise guide to pharmacology 2017/18: ligand-gated ion channels. British Journal of Pharmacology 174:S130—S159.

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[12] Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Mennti, F.S., Vance, K.M., Ogden, K.K., et al., 2010. Glutamate receptor ion channels: structure, regulation, and function. Pharmacological Reviews 62:405–496.

[13] Marquard, J., Otter, S., Welters, A., Stirban, A., Fischer, A., Eglinger, J., et al., 2015. Characterization of pancreatic NMDA receptors as possible drug targets for diabetes treatment. Nature Medicine 21:363–372.

[14] Marquard, J., Stirban, A., Schliess, F., Sievers, F., Welters, A., Otter, S., et al., 2016. Effects of dextromethorphan as add-on to sitagliptin on blood glucose and serum insulin concentrations in individuals with type 2 diabetes mellitus: a randomized, placebo-controlled, double-blinded, multiple crossover, single-dose clinical trial. Diabetes Obesity and Metabolism 18:100–103.

[15] Wollheim, C.B., Maechler, P., 2015. Beta cell glutamate receptor antagonists: novel oral antidiabetic drugs? Nature Medicine 21:310.

[16] Mitani, H., Shirayama, Y., Yamada, T., Maeda, K., Ashby Jr., C.R., Kawahara, R., 2006. Correlation between plasma levels of glutamate, alanine and serine with severity of depression. Progress in Neuropsychopharmacology and Biological Psychiatry 30:1155–1158.

[17] Bettler, B., Collingridge, G.L., Dingledine, R., Heinemann, S.F., Hollmann, M., Lerma, J., et al., 2019. Ionotropic glutamate receptors (version 2019.4) in the IUPHAR/BPS Guide to Pharmacology Database. IUPHAR/BPS Guide to Pharmacology 2019(4):1155–1158.

[18] Cabrera, O., Jacques-Silva, M.C., Speier, S., Yang, S.N., Kohler, M., Fachado, A., et al., 2008. Glutamate is a positive autocrine signal for glucagon release. Cell Metabolism 7:545–554.

[19] Cho, J.H., Chen, L., Kim, M.H., Chow, R.H., Hille, B., Koh, D.S., 2010. Characteristics and functions of {alpha}-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors expressed in mouse pancreatic {alpha}-cells. Endocrinology 151:1541–1550.

[20] Muroyama, A., Uehara, S., Yatsushiro, S., Echigo, N., Morimoto, R., Morita, M., et al., 2004. A novel variant of ionotropic glutamate receptor regulates somatostatin secretion from delta-cells of islets of Langerhans. Diabetes 53:1743–1753.

[21] Wu, Z.Y., Zhu, L.J., Zou, N., Bombek, L.K., Shao, C.Y., Wang, N., et al., 2012. AMPA receptors regulate exocytosis and insulin release in pancreatic beta cells. Traffic 13:1124–1139.

[22] Bertrand, G., Gross, R., Puech, R., Loubatieres-Mariani, M.M., Bockaert, J., 1993. Glutamate stimulates glucagon secretion via an excitatory amino acid receptor of the AMPA subtype in rat pancreas. European Journal of Pharmacology 237:45–50.

[23] Briant, L.J.B., Reinbothe, T.M., Spiliotis, I., Miranda, C., Rodriguez, B., Rorsman, P., 2018. delta-cells and beta-cells are electrically coupled and regulate alpha-cell activity via somatostatin. Journal of Physiology 596:197–215.

[24] Taborsky Jr., G.J., Smith, P.H., Porte Jr., D., 1979. Differential effects of somatostatin analogues on alpha- and beta-cells of the pancreas. American Journal of Physiology 236:E123–E128.

[25] Bertrand, G., Gross, R., Puech, R., Loubatieres-Mariani, M.M., Bockaert, J., 1992. Evidence for a glutamate receptor of the AMPA subtype which mediates insulin release from rat perfused pancreas. British Journal of Pharmacology 106:354–359.

[26] Segerstolpe, A., Palasantza, A., Eliasson, P., Andersson, E.M., Andreasson, A.C., Sun, X., et al., 2016. Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. Cell Metabolism 24:593–607.. http://sandberg.cmb.ki.se/pancreas/.

[27] Mulle, C., Sailer, A., Swanson, G.T., Brana, C., O'Gorman, S., Bettler, B., et al., 2000. Subunit composition of kainate receptors in hippocampal interneurons. Neuron 28:475–484.

[28] Contractor, A., Mulle, C., Swanson, G.T., 2011. Kainate receptors coming of age: milestones of two decades of research. Trends in Neurosciences 34:154–163.

[29] Perrais, D., Veran, J., Mulle, C., 2010. Gating and permeation of kainate receptors: differences unveiled. Trends in Pharmacological Sciences 31:516–522.

[30] Ruiz, A., Sachidhanandam, S., Utvik, J.K., Coussen, F., Mulle, C., 2005. Distinct subunits in heteromeric kainate receptors mediate ionotropic and metabotropic function at hippocampal mossy fiber synapses. Journal of Neuroscience 25:11710–11718.

[31] Crepel, V., Mulle, C., 2015. Physiopathology of kainate receptors in epilepsy. Current Opinion in Pharmacology 20:83–88.

[32] Mulle, C., Sailer, A., Perez-Otano, I., Dickinson-Anson, H., Castillo, P.E., Bureau, I., et al., 1998. Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. Nature 392:601–605.

[33] Micheau, J., Vimeney, A., Normand, E., Mulle, C., Riedel, G., 2014. Impaired hippocampus-dependent spatial flexibility and sociability represent autism-like phenotypes in GluK2 mice. Hippocampus 24:1059–1069.

[34] Falcón-Moya1, R., Sihra, T.S., Rodríguez-Moreno, A., 2018. Kainate receptors: role in epilepsy. Frontiers in Molecular Neuroscience 11.

[35] Bureau, I., Bischoff, S., Heinemann, S.F., Mulle, C., 1999. Kainate receptor-mediated responses in the CA1 field of wild-type and GluR6-deficient mice. Journal of Neuroscience 19:653–663.

[36] Sachidhanandam, S., Blanchet, C., Jeantet, Y., Cho, Y.H., Mulle, C., 2009. Kainate receptors act as conditional amplifiers of spike transmission at hippocampal mossy fiber synapses. Journal of Neuroscience 29:5000–5008.

[37] Flurkey, K., Currer, J.M., Harrison, D.E., 2007. The mouse in aging research. In: Fox, J.G. (Ed.), The mouse in biomedical Research, 2nd ed. Burlington: Elsevier. p. 637–72.

[38] Lamy, C.M., Sanno, H., Labouebe, G., Picard, A., Magnan, C., Chatton, J.Y., et al., 2014. Hypoglycemia-activated GLUT2 neurons of the nucleus tractus solitarius stimulate vagal activity and glucagon secretion. Cell Metabolism 19:527–538.

[39] Roger, B., Papin, J., Vacher, P., Raoux, M., Mulot, A., Dubois, M., et al., 2011. Adenylyl cyclase 8 is central to glucagon-like peptide 1 signalling and effects of chronically elevated glucose in rat and human pancreatic beta cells. Diabetologia 54:390–402.

[40] Perrier, R., Pirog, A., Jaffredo, M., Gaitan, J., Catargi, B., Renaud, S., et al., 2018. Bioelectronic organ-based sensor for microfluidic real-time analysis of the demand in insulin. Biosensors and Bioelectronics 117:253–259.

[41] Karaca, M., Castel, J., Tourrel-Cuzin, C., Brun, M., Geant, A., Dubois, M., et al., 2009. Exploring functional beta-cell heterogeneity in vivo using PSA-NCAM as a specific marker. PLoS One 4:e5555.

[42] Campana, M., Bellini, L., Rouch, C., Rachdi, L., Coant, N., Butin, N., et al., 2018. Inhibition of central de novo ceramide synthesis restores insulin signaling in hypothalamus and enhances beta-cell function of obese Zucker rats. Molecular Metabolism 8:23–36.

[43] Karaca, M., Frigerio, F., Migrenne, S., Martin-Levilain, J., Skytt, D.M., Pajecka, K., et al., 2015. GDH-dependent glutamate oxidation in the brain dictates peripheral energy substrate distribution. Cell Reports 13:365–375.

[44] Bornat, Y., Raoux, M., Boutaib, Y., Morin, F.O., Charpentier, G., Lang, J., et al., 2010. Detection of electrical activity of pancreatic ß-cells using micro-electrode arrays. In: Proceedings 5th IEEE int. Symposium on electronic design. Test & Applications.

[45] Lang, J., Catargi, B., Renaud, S., Raoux, M., Charpentier, G., Bornat, Y., 2010. Sensor for measuring the activity of beta-pancreatic cells or of islets of Langerhans, manufacture and use of such a sensor. WO2011086105A1.

[46] Lebreton, F., Pirog, A., Belouah, I., Bosco, D., Berney, T., Meda, P., et al., 2015. Slow potentials encode intercellular coupling and insulin demand in pancreatic beta cells. Diabetologia 58:1291–1299.

[47] Gut, I.M., Beske, P.H., Hubbard, K.S., Lyman, M.E., Hamilton, T.A., McNutt, P.M., 2013. Novel application of stem cell-derived neurons to evaluate the time- and dose-dependent progression of excitotoxic injury. PLoS One 8:e64423.

[48] Copits, B.A., Swanson, G.T., 2013. Kainate receptor post-translational modifications differentially regulate association with 4.1n to control activity-dependent receptor endocytosis. Journal of Biological Chemistry 288:8952–8965.

[49] Hughey, C.C., Wasserman, D.H., Lee-Young, R.S., Lantier, L., 2014. Approach to assessing determinants of glucose homeostasis in the conscious mouse. Mammalian Genome 25:522—538.

[50] Bloom, S.R., Edwards, A.V., Hardy, R.N., 1978. The role of the autonomic nervous system in the control of glucagon, insulin and pancreatic polypeptide release from the pancreas. Journal of Physiology 280:9—23.

[51] N'Guyen, J.M., Magnan, C., Laury, M.C., Thibault, C., Leveteau, J., Gilbert, M., et al., 1994. Involvement of the autonomic nervous system in the in vivo memory to glucose of pancreatic beta cell in rats. Journal of Clinical Investigation 94:1456—1462.

[52] Yamatani, K., Ohnuma, H., Niijima, A., Igarashi, M., Sugiyama, K., Daimon, M., et al., 1998. Impaired vagus nerve-mediated control of insulin secretion in Wistar fatty rats. Metabolism 47:1167—1173.

[53] Pedraza, E., Karajic, A., Raoux, M., Perrier, R., Pirog, A., Lebreton, F., et al., 2015. Guiding pancreatic beta cells to target electrodes in a whole-cell biosensor for diabetes. Lab Chip 15:3880—3890.

[54] Raoux, M., Bornat, Y., Quotb, A., Catargi, B., Renaud, S., Lang, J., 2012. Non-invasive long-term and real-time analysis of endocrine cells on micro-electrode arrays. Journal of Physiology 590:1085—1091.

[55] Jaffredo, M., Pirog, A., Bertin, E., Catargi, B., Renaud, S., Lang, J., et al., 2018. Differential beta cell coupling patterns drive biphasic activity. Diabetologia 61: S17—S18.

[56] Gerich, J.E., Charles, M.A., Grodsky, G.M., 1974. Characterization of the effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas. Journal of Clinical Investigation 54:833—841.

[57] Curry, D.L., Morris, J.G., Rogers, Q.R., Stern, J.S., 1982. Dynamics of insulin and glucagon secretion by the isolated perfused cat pancreas. Comparative Biochemistry and Physiology A Comparative Physiology 72:333—338.

[58] Munoz, A., Hu, M., Hussain, K., Bryan, J., Aguilar-Bryan, L., Rajan, A.S., 2005. Regulation of glucagon secretion at low glucose concentrations: evidence for adenosine triphosphate-sensitive potassium channel involvement. Endocrinology 146:5514—5521.

[59] Yu, Q., Shuai, H., Ahooghalandari, P., Gylfe, E., Tengholm, A., 2019. Glucose controls glucagon secretion by directly modulating cAMP in alpha cells. Diabetologia 62:1212—1224.

[60] Roh, E., Song, D.K., Kim, M.-S., 2016. Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism. Experimental and Molecular Medicine 48:e216.

[61] Yuan, H., Low, C.M., Moody, O.A., Jenkins, A., Traynelis, S.F., 2015. Ionotropic GABA and Glutamate receptor mutations and human neurologic diseases. Molecular Pharmacology 88:203—217.

[62] Chandra, N., Awasthi, R., Ozdogan, T., Johenning, F.W., Imbrosci, B., Morris, G., et al., 2019. A cellular mechanism underlying enhanced capability for complex olfactory discrimination learning. eNeuro 6.

[63] Mundinger, T.O., Taborsky Jr., G.J., 2012. Minireview: the role of the autonomic nervous system in mediating the glucagon response to hypoglycemia. Endocrinology 153:1055—1062.

[64] Tong, Q., Ye, C., McCrimmon, R.J., Dhillon, H., Choi, B., Kramer, M.D., et al., 2007. Synaptic glutamate release by ventromedial hypothalamic neurons is part of the neurocircuitry that prevents hypoglycemia. Cell Metabolism 5:383—393.

[65] Xu, H., Smith, B.N., 2015. Presynaptic ionotropic glutamate receptors modulate GABA release in the mouse dorsal motor nucleus of the vagus. Neuroscience 308:95—105.

[66] Shaltiel, G., Maeng, S., Malkesman, O., Pearson, B., Schloesser, R.J., Tragon, T., et al., 2008. Evidence for the involvement of the kainate receptor subunit GluR6 (GRIK2) in mediating behavioral displays related to behavioral symptoms of mania. Molecular Psychiatry 13:858—872.

[67] Uehara, S., Muroyama, A., Echigo, N., Morimoto, R., Otsuka, M., Yatsushiro, S., et al., 2004. Metabotropic glutamate receptor type 4 is involved in autoinhibitory cascade for glucagon secretion by alpha-cells of islet of Langerhans. Diabetes 53:998—1006.

[68] Valbuena, S., Lerma, J., 2016. Non-canonical signaling, the hidden life of ligand-gated ion channels. Neuron 92:316—329.

[69] Negrete-Díaz, J.V., Sihra, T.S., Flores, G., Rodríguez-Moreno, A., 2018. Non-canonical mechanisms of presynaptic kainate receptors controlling glutamate release. Frontiers in Molecular Neuroscience 11.

[70] Rozas, J.L., Paternain, A.V., Lerma, J., 2003. Noncanonical signaling by ionotropic kainate receptors. Neuron 39:543—553.

[71] Cordoba, M., Rodriguez, S., Gonzalez Moron, D., Medina, N., Kauffman, M.A., 2015. Expanding the spectrum of Grik2 mutations: intellectual disability, behavioural disorder, epilepsy and dystonia. Clinical Genetics 87:293—295.

[72] Motazacker, M.M., Rost, B.R., Hucho, T., Garshasbi, M., Kahrizi, K., Ullmann, R., et al., 2007. A defect in the ionotropic glutamate receptor 6 gene (GRIK2) is associated with autosomal recessive mental retardation. The American Journal of Human Genetics 81:792—798.

[73] Lau, J.C., Kroes, R.A., Moskal, J.R., Linsenmeier, R.A., 2013. Diabetes changes expression of genes related to glutamate neurotransmission and transport in the Long-Evans rat retina. Molecular Vision 19:1538—1553.

[74] Lin, H.J., Huang, Y.C., Lin, J.M., Wu, J.Y., Chen, L.A., Tsai, F.J., 2013. Association of genes on chromosome 6, GRIK2 , TMEM217 and TMEM63B (linked to MRPL14) with diabetic retinopathy. Ophthalmologica 229:54—60.

[75] Gelling, R.W., Du, X.Q., Dichmann, D.S., Romer, J., Huang, H., Cui, L., et al., 2003. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. Proceedings of the National Academy of Sciences of the United States of America 100:1438—1443.

[76] Lee, Y., Wang, M.Y., Du, X.Q., Charron, M.J., Unger, R.H., 2011. Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. Diabetes 60:391—397.

[77] Winzell, M.S., Brand, C.L., Wierup, N., Sidelmann, U.G., Sundler, F., Nishimura, E., et al., 2007. Glucagon receptor antagonism improves islet function in mice with insulin resistance induced by a high-fat diet. Diabetologia 50:1453—1462.

[78] Hancock, A.S., Du, A., Liu, J., Miller, M., May, C.L., 2010. Glucagon deficiency reduces hepatic glucose production and improves glucose tolerance in adult mice. Molecular Endocrinology 24:1605—1614.

[79] Traub, S., Meier, D.T., Schulze, F., Dror, E., Nordmann, T.M., Goetz, N., et al., 2017. Pancreatic alpha cell-derived glucagon-related peptides are required for beta cell adaptation and glucose homeostasis. Cell Reports 18:3192—3203.

[80] De Tata, V., 2014. Age-Related impairment of pancreatic beta-cell function: pathophysiological and cellular mechanisms. Frontiers in Endocrinology 5.

[81] Mollerud, S., Frydenvang, K., Pickering, D.S., Kastrup, J.S., 2017. Lessons from crystal structures of kainate receptors. Neuropharmacology 112:16—28.