

Glucose-sensing neurons: Are they physiologically relevant?

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Abstract

Glucose homeostasis is of paramount concern to the brain since glucose is its primary fuel. Thus, the brain has evolved mechanisms to sense and respond to changes in glucose levels. The efferent aspects of the central nervous system response to hypoglycemia are relatively well understood. In addition, it is accepted that the brain regulates food intake and energy balance. Obesity and diabetes both result from and cause alterations in the central nervous system function. Thus, it is reasonable to hypothesize that the brain also regulates daily glucose homeostasis and energy balance. However, little is known about how the brain actually senses and responds to changes in extracellular glucose. While there are neurons in the brain that change their action potential frequency in response to changes in extracellular glucose, most studies of these neurons have been performed using glucose levels that are outside the physiologic range of extracellular brain glucose. Thus, the physiologic relevance of these glucose-sensing neurons is uncertain. However, recent studies show that glucose-sensing neurons do respond to physiologic changes in extracellular glucose. This review will first investigate the data regarding physiologic glucose levels in the brain. The various subtypes of physiologically relevant glucose-sensing neurons will then be discussed. Based on the relative glucose sensitivity of these subtypes of glucose-sensing neurons, possible roles in the regulation of glucose homeostasis are hypothesized. Finally, the question of whether these neurons are only glucose sensors or whether they play a more integrated role in the regulation of energy balance will be considered. Published by Elsevier Science Inc.

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1. Introduction

Glucose homeostasis is of critical concern to the brain, since glucose is its primary fuel. Either a large rise or fall in plasma glucose levels causes the brain to activate the sympathetic nervous system [1–3]. Thus, the brain responds to large changes in plasma glucose and initiates compensatory responses to maintain glucose homeostasis. Both central and peripheral glucose sensors may serve to inform the brain that plasma glucose levels are falling to dangerous levels. Much less is known about the mechanisms that sense an increase of plasma glucose. The ventromedial region of the hypothalamus (VMH), which contains both the arcuate nucleus (ARC) and the ventromedial hypothalamic nucleus (VMN), plays an important role in the response to hypoglycemia [4,5]. Recent evidence indicates that the brainstem may play a role [6–8]. In addition, glucose sensors exist in

the portal vein [9–11]. Information from these peripheral glucose sensors is then relayed to the brain via portal afferents [12]. Glucose sensors are also present in the carotid body [13]. While the efferent mechanisms maintaining glucose homeostasis are fairly well characterized [14], little is known about if and how the brain actually senses and responds to large changes in plasma glucose levels. Finally, the brain is implicated in the regulation of feeding and energy balance [15]. Thus, it is reasonable to hypothesize that the brain also senses smaller perturbations of plasma glucose, which enable it to regulate meal initiation and termination.

For many years, we have known that glucose regulates neuronal activity. For example, in 1964, two separate laboratories described neurons within the hypothalamus, which change their action potential frequency in response to changes in plasma glucose [16,17]. Furthermore, glucose infusion into the portal vein alters the action potential frequency of neurons in the lateral hypothalamus (LH) [10]. Later studies showed that direct application of glucose

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increased the action potential frequency of ‘glucose-responsive’ or GR neurons, while decreasing the action potential frequency of ‘glucose-sensitive’ or GS neurons. GR neurons are most abundant in the VMH. In contrast, GS neurons are most common in the LH [18]. The VMN and LH are believed to be reciprocally related in the regulation of food intake and energy balance [16,17]. Twenty-five years after these initial descriptions, Ashford et al. [19] showed that GR neurons utilize the ATP-sensitive K^+ (KATP) channel to sense glucose. That is, similar to the pancreatic β -cell, rising glucose levels increase the intracellular ATP to ADP ratio and close the KATP channel. This depolarizes the β -cell and activates voltage-sensitive calcium channels that mediate insulin secretion [20]. However, glucose-initiated depolarization of GR neurons increases action potential frequency. Less is known about the mechanism by which GS neurons sense glucose, although decreased activity of the Na^+/K^+ ATPase with decreased ATP has been suggested [21,22]. Presumably, decreased activity of the Na^+/K^+ ATPase would cause depolarization and increase action potential frequency [22].

While it is attractive to hypothesize that GR and GS neurons play a role in glucose homeostasis, it is important to note that the majority of studies of these neurons used glucose levels outside the physiologic range (0–10 or 20 mM) [22–27]. A cerebrospinal fluid level of 0 mM glucose would be incompatible with life. In fact, most neurons throughout the brain become silent when glucose levels fall below 1 mM for an extended time [28]. Moreover, virtually all studies of the central nervous system use 10 mM glucose in the solutions that bathe neurons. This assumes that the extracellular fluid levels of glucose in the brain equal those of the plasma. It is noteworthy that even if this were true, 10 mM plasma glucose is not euglycemia (~5 mM) but rather hyperglycemia. However, recent evidence demonstrates that brain glucose concentrations never achieve that of plasma [29–32]. Therefore, before evaluating the role of glucose-sensing neurons in glucose homeostasis, we must first consider the physiologic levels of extracellular glucose in the brain.

2. What are physiologic levels for extracellular glucose in the brain?

Surprisingly, despite significant study the concentration of extracellular glucose in the brain is still controversial. While there are several *in vivo* methods for measurement of brain glucose levels, each has limitations. These methods include glucose electrodes, microdialysis and magnetic resonance spectroscopy (MRS). Glucose electrodes allow for ‘real-time’ measurement of changes in extracellular brain glucose levels in response to changing plasma levels in discrete brain nuclei. However, one risks overestimating glucose concentration because these electrodes use glucose oxidase to detect glucose. This enzyme also oxidizes ascorbate and uric acid,

both of which are present in the brain [21,32]. Measurement of extracellular glucose levels by microdialysis employs the method of zero net flux in which net concentration change of glucose across the dialysis membrane is plotted against the perfusate glucose concentration. The point at which there is no concentration change or ‘zero net flux’ indicates the extracellular glucose concentration in the brain. This technique provides sensitivity at the expense of temporal resolution [30]. Finally, MRS utilizes proton resonance spectra for noninvasive quantitation of glucose levels at the expense of spatial resolution [33,34].

Methodological limitations notwithstanding, there is convincing evidence that extracellular glucose levels in most regions of the brain are much lower than those in the plasma. Extracellular glucose levels during plasma euglycemia are reported to range from 0.3 to 3.3 mM [29–31,34–40]. Silver and Erecinska [21,32] provided the only data in which extracellular brain glucose levels were simultaneously measured as plasma glucose levels were manipulated. In their studies, Silver and Erecinska clamped peripheral glucose and measured extracellular brain glucose using a glucose oxidase electrode. Extracellular glucose in the brain was about 2.5 mM in a fed, anesthetized rat whose plasma glucose was 7.6 mM. This plasma level is on the high side of euglycemia. Thus, plasma levels in the euglycemic range of 5–8 mM correspond to brain levels of about 1–2.5 mM (see Fig. 1). This is comparable to microdialysis measurements of 0.7–1.2 mM brain glucose when plasma glucose levels were about 5 mM [30] (E.C. McNay, personal communication). When plasma levels fell to 2–3 mM during insulin-induced hypoglycemia, brain levels fell to approximately 0.5 mM. Raising plasma glucose levels to those seen after a large meal (15–17 mM) resulted in brain levels of 4.5 mM [32].

As mentioned earlier, there is a wide range of *in vivo* extracellular glucose levels reported in the literature. Different methodology may contribute to this variability. In

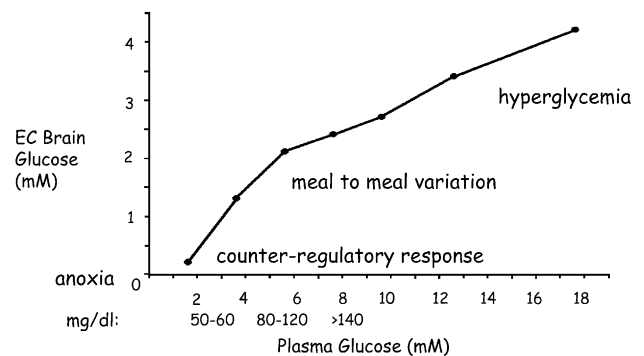


Fig. 1. Extracellular (EC) brain glucose levels versus plasma glucose levels. Plasma glucose levels of about 2–3 mM (50–60 mg/dl) correlate with those seen during initiation of the counterregulatory response. Plasma levels of about 5–8 (80–120 mg/dl) are seen during euglycemia and are related to levels seen during meal to meal variation. Plasma glucose levels over 8 mM or 140 mg/dl are seen during hyperglycemia. Brain glucose levels near 0 mM might be observed during anoxia. Adapted from Ref. [21].

addition, differences in strain of rat and brain region may also be important variables. Microdialysis studies using the zero net flux method show that glucose levels vary with brain region and strain [30,31]. Even more interesting, brain glucose levels change with activity in a region-specific fashion [29]. McNay et al. show that steady state extracellular glucose levels in the hippocampus of awake adult Sprague–Dawley rats are approximately 1 mM, whereas adult and aged Fischer 344 rats have significantly higher hippocampal glucose levels of 1.2 mM [30]. In contrast, striatal glucose levels in conscious Sprague–Dawley rats are only about 0.7 mM. Plasma glucose levels were approximately 5 mM in these studies (E.C. McNay, personal communication). Interestingly, while hippocampal glucose levels fell by 32% during a test of spatial memory in adult Sprague–Dawley rats, striatal levels were unaffected. When glucose was injected peripherally, performance was improved and hippocampal levels were not decreased. Moreover, striatal levels were unchanged, indicating a dissociation between brain and plasma glucose levels in specific regions of the brain [29,31].

Regardless of the variability in exact measurement of extracellular glucose levels in the brain, it is clear that extracellular glucose levels of either 0 or 10–20 mM are nonphysiologic. That is, brain extracellular glucose levels during plasma euglycemia (5–8 mM) probably range between 0.7 and 2.5 mM [21,30,32]. Brain glucose levels plateau at approximately 5 mM under severe plasma hyperglycemia. On the other hand, brain levels of approximately 0.2–0.5 mM correlate with the 2–3-mM plasma glucose levels, which trigger counterregulation (Fig. 1) [32]. In light of this work, most studies of both glucose-sensing and non-glucose-sensing neurons are being performed at glucose levels outside the physiologic range. This issue is of fundamental importance not only to scientists studying food intake and metabolism, but also to those investigating seemingly nonrelated topics. As seen from the above data, neuronal activity in the presence of 10 mM glucose is significantly different than at the 0.7–2.5-mM extracellular brain glucose levels associated with plasma euglycemia. This will be discussed in greater detail below. Before leaving the topic of physiologic glucose levels in the brain, one caveat must be mentioned. If the neurons under investigation are in or near a region lacking a blood–brain barrier, brain glucose levels may approximate plasma levels. Neurons in these regions may be normally exposed to higher levels of extracellular glucose. However, under physiologic conditions, the majority of neurons are unlikely to be exposed to extracellular glucose levels above 5 mM.

3. Hypothalamic glucose-sensing neurons respond to physiologic changes in extracellular glucose in vitro

In addition to measuring extracellular brain glucose levels, Silver and Erecinska [21] also recorded action

potential frequencies of VMN and LH neurons in response to changes in extracellular glucose. They found that 33% of LH neurons were sensitive to small changes in glucose. In agreement with previous studies [22], increased plasma glucose inhibited the majority of LH glucose-sensing neurons. These neurons were randomly distributed throughout the LH and exhibited heterogeneous responses to changes in plasma glucose. Type 1 neurons were maximally active when plasma glucose levels were 5.6 mM (brain glucose 2.1) and were completely silent when plasma levels rose to 10–12 mM (brain glucose 3.2–3.4). Type 1 neurons were specifically glucose sensitive and did not respond to other stimuli such as light pinch or cold application to the tail of the rat. Neurons of Type 2 and Type 3 were only inhibited by plasma glucose levels of 17 mM and higher (brain glucose >4.2 mM). These subclasses of LH glucose-sensing neurons responded to other stimuli (pinch and/or cold) in addition to glucose. Type 2 and Type 3 neurons were also responsive to a larger range of glucose concentrations, with Type 3 reaching maximal activity when brain glucose levels fell below 0.6 mM. Only 5–7% of LH neurons (Type 4) increased their action potential frequency rate when blood glucose increased beyond 7 mM. In the VMH, however, 43% of neurons increased their action potential frequency as glucose levels increased. These neurons were not sensitive to any other stimuli. Most of these glucose-sensing VMH neurons were silent at plasma glucose levels below 3–4 mM and progressively increased their action potential frequency as plasma glucose levels increased to 15 mM or higher. Hyperglycemia failed to inhibit the action potential frequency of VMH neurons. Thus, the elegant work of Silver and Erecinska [21] shows that VMH and LH neurons in an anesthetized rat are sensitive to small changes in plasma glucose. However, they do not clarify the question of whether these glucose-sensing neurons themselves directly sense small changes in extracellular brain glucose level.

Our lab has recently characterized hypothalamic glucose-sensing neurons whose action potential frequency changes in response to changes of extracellular brain glucose from a steady state level of 2.5 mM derived from the work of Silver and Erecinska [21,32,41]. These studies were performed on brain slices using patch clamp recording. We found that VMN glucose-sensing neurons do respond to physiologically relevant changes in extracellular glucose. Moreover, glucose sensing in the VMN involves a complex convergence of pre- and postsynaptic mechanisms (Fig. 2). There are two subtypes of VMN glucose-sensing neurons that respond directly to a decrease in extracellular glucose. Glucose-excited (GE) neurons increase their action potential frequency as extracellular glucose increases from 0.1 to 2.5 mM [41]. Our preliminary data indicate that GE neurons respond gradually to a linear change in extracellular glucose from 0.1 to 2.5 mM at which point the response to glucose plateaus; their EC_{50} for glucose is 0.64 mM (unpublished observation). It has been reported that all neurons in the brain, glucose sensing or otherwise, are silent when glucose

SUBTYPES OF VMN GLUCOSENSING NEURONS

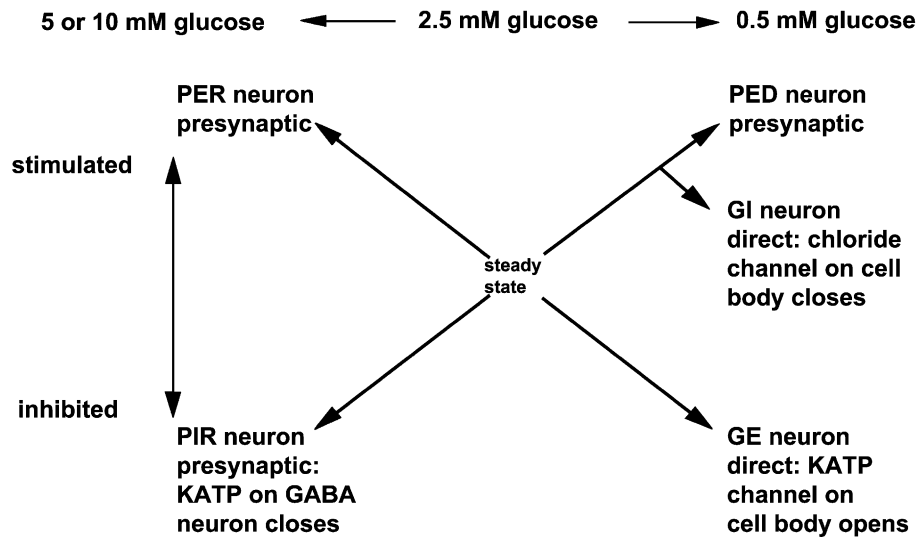


Fig. 2. Schematic overview of the subtypes of VMN glucose-sensing neurons and mechanisms by which they sense glucose. Glucose concentration increases from right to left and neuronal activity increases from bottom to top of the diagram. Mechanism is listed below neuronal subtype. Thus, PED and GI neurons are shown to the upper right indicating that they are excited, presynaptically or via closure of a chloride channel, respectively, as extracellular glucose levels decrease. GE neurons are shown on the lower right indicating that they are inhibited by decreased extracellular glucose directly via opening of a KATP channel. PIR neurons are shown on the lower left portion, indicating that they are inhibited as extracellular glucose levels are raised (presynaptically via closure of a KATP channel on a GABA neuron) and PER neurons are shown on the upper left indicating that they are (presynaptically) excited as extracellular glucose is raised. Adapted from Ref. [41].

levels fall below 1 mM [28]. We have found that this is true only if neurons are exposed to extremely low glucose for more than 12–15 min. In fact, exposure to 0.1 mM glucose for over 15 min irreversibly damages most neurons regardless of whether they are glucose sensors or not (unpublished observation). However, only VMN GE neurons decrease their action potential frequency when exposed to extracellular glucose levels below 1 mM for less than 10 min. Non-glucose-sensing VMN neurons sustain very high firing rates in 0.1 mM glucose for up to 10–12 min. Finally, GE neurons are sensitive to changes in extracellular glucose as small as 100 μ M (unpublished observation). These GE neurons are similar to the GR neurons described earlier in that they respond to glucose via the KATP channel [41]. On the other hand, glucose-inhibited (GI) neurons decrease their action potential frequency as glucose levels increase from 0.1 to 2.5 mM, where the neurons are completely silent [41]. Although these neurons appear to respond in a similar fashion as GS neurons, we do not believe that these are the same class of neurons for the following reasons. It has been suggested that GS neurons sense glucose via changes in the Na^+/K^+ ATPase pump [21,22]. If this were the case for VMN GI neurons, then the current voltage relations in 0.1 and 2.5 mM glucose should be parallel. However, the response to decreased extracellular glucose for GI neurons reversed at the chloride equilibrium potential [41]. This suggests that a chloride channel is involved in their glucose-sensing mechanism. There are chloride channels that are

sensitive to intracellular ATP levels. These include the cystic fibrosis transmembrane regulator chloride conductance (CFTR) [42]. Interestingly, CFTR channels are also members of the ATP binding cassette superfamily and are sensitive to sulfonylurea drugs [43]. Sulfonylureas (SUR) close the CFTR channel while ATP opens it [44]. This is consistent with our observation that a decrease in ATP closes a chloride channel in these neurons causing them to depolarize and increase their action potential frequency. Tolbutamide excites GI neurons which is also consistent with a CFTR-like channel [41]. CFTR channel gene, mRNA and protein are expressed in the human and rat brain, as well as in hypothalamic cell lines [45–47]. However, these channels have not yet been found on individual glucose-sensing neurons.

Three other subtypes of physiologically relevant VMN glucose-sensing neurons are presynaptically modulated by glucose [41]. Although these VMN neurons are not intrinsically glucose sensing but rather modulated by glucose-controlled synaptic input, we consider them in the overall category of VMN glucose-sensing neurons. Of these, one subtype is presynaptically excited as extracellular glucose levels fall below 2.5 mM. We refer to these as presynaptically excited by decreased glucose or PED neurons. The other two subtypes of presynaptically modulated glucose-sensing neurons respond to an increase in extracellular glucose from 2.5 to 5 or 10 mM. PER neurons are presynaptically excited as glucose levels are raised to

5 mM, while PIR neurons are inhibited. The presynaptic mechanisms by which PED and PER neurons sense glucose are unclear. However, PIR neurons appear to receive glucose-controlled inhibitory input from γ -aminobutyric acid (GABA)-ergic neurons as glucose levels rise. Moreover, tolbutamide mimics the effect of increased glucose on PIR neurons, suggesting the involvement of a KATP channel. We hypothesize that this KATP channel is located on the terminal of the presynaptic GABAergic neuron, which synapses on the PIR neuron. Such presynaptic channels have been described in the substantia nigra where glucose has been shown to regulate GABA release [48,49].

The interactions between pre- and postsynaptic influences are quite complex. Interestingly, some directly glucose-sensing VMN neurons are also influenced by other glucose-sensing neurons. We have often observed a GE neuron receiving inhibitory glucose-controlled presynaptic input in response to increased extracellular glucose [41]. This leads to a biphasic response to changes in extracellular glucose in which the GE neuron is inhibited by a deviation in either direction from 2.5 mM. In this case, the overall effect of tolbutamide is excitatory, suggesting that the closure of the somal KATP channel on the GE neuron itself has a greater effect on neuronal activity than does closing the presynaptic KATP channel. The KATP channel is an octomeric complex consisting of two receptors for SUR and four pore-forming units for K^+ (Kir 6.2 or 6.1) [50]. There are at least two types of SUR, SUR1 and SUR2 (A and B), with differing sensitivities to sulfonylureas. These subtypes of SURs appear to be differentially expressed on cell bodies and terminals [51], allowing for differential glucose sensitivity of cell bodies versus terminals [52,53]. Biphasic responses have also been observed for GI neurons. Finally, VMN neurons receiving glucose-controlled presynaptic input in response to decreased glucose also receive glucose-controlled presynaptic input in response to increased glucose [41]. Thus, both GI neurons and these latter neurons are quiescent at steady state levels of 2.5 mM extracellular glucose and excited when glucose levels either increase or decrease. This further supports our hypothesis that GI neurons are not the same class of neurons as GS neurons, which are stimulated as glucose levels decrease from 10 or 20 to 0 mM. That is, while the GS neurons studied by Oomura et al. [17] were quiet at 10 or 20 mM, ours are active due to presynaptic excitation. It is important to note that the observed presynaptic contribution to glucose sensing is highly dependent on the tissue preparation studied. Obviously, these effects will not be observed in studies using dissociated neurons. Moreover, in our slice preparations, extrahypothalamic neurons which project to the VMN are not intact. This includes input from important regions such as the amygdala and brainstem. While some glucose-sensing input to the VMN may occur on nerve terminals of neurons projecting from these regions, somal glucose sensing is most likely involved as well. This may explain some of the discrepancies between our data and those obtained in vivo where all presynaptic input is intact.

Finally, it is possible that glucose-sensing neurons in different regions of the brain may behave differently from those in the VMN. In point, the VMN contains neurons that are directly responsive to decreased extracellular glucose, but it does not contain neurons that directly sense increased extracellular glucose. Yet, VMN neurons receive glucose-controlled presynaptic input in response to increased extracellular glucose. This suggests that there are inherent differences in glucose sensitivity between VMN and non-VMN neurons. Furthermore, glucose-sensing neurons in different parts of the brain may be exposed to a different extracellular milieu (e.g., differing neurotransmitter complement or level). There are many mechanisms (e.g., phosphorylation) by which this might result in differences in glucose-sensing properties.

4. Putative roles for glucose-sensing neurons

As mentioned previously, glucose is the primary fuel of the brain. Thus, the brain has a vested interest in maintaining extracellular glucose levels within a satisfactory range. There are two separate components of this goal. First, during severe energy depletion (e.g., anoxia and ischemia), individual neurons must protect themselves from the resultant cytotoxic release of glutamate [54]. A second and totally separate issue relates to the need to ensure that peripheral metabolism and glucose production are sufficient to meet the needs of the brain. This involves integration among information regarding local extracellular glucose levels within the brain, information from glucose sensors in the periphery and information regarding overall metabolic status in order to generate a coordinated regulation of glucose homeostasis. It is the opinion of this author that this second function is truly 'glucose sensing', while the former is simply neuroprotection. It is important to distinguish between the requirements for these two functions in order to conclude that glucose-sensing neurons are involved in the maintenance of glucose homeostasis. Neuroprotective versus glucose-sensing functions and requirements are discussed in more detail below.

4.1. Neuroprotection

Virtually all neurons possess KATP channels [55]. In order for hypothalamic neurons to function specifically as glucose sensors that regulate glucose homeostasis, there must be a functional difference between KATP-channel-dependent GE neurons in the hypothalamus and KATP-containing neurons elsewhere in the brain. When glucose levels fall extremely low, as during anoxia and stroke, neurons are endangered due to massive cytotoxic glutamate release. The cytotoxic effects of glutamate are partly reduced by K^+ channel openers and exacerbated by SUR [54]. Thus, when ATP levels are extremely low, KATP channels open and hyperpolarize the neuron. This prevents

glutamatergic excitation that leads to cell death. Additionally, differential expression of KATP channel subunits is correlated with differential sensitivity to metabolic inhibition [53]. Dopaminergic neurons from wild-type mice coexpress both SUR1+Kir6.2 and SUR2B+Kir6.2. The combination of SUR1+Kir6.2, but not SUR2B+Kir6.2, produces KATP channels with a high sensitivity to metabolic inhibition induced by rotenone. In homozygous weaver mice, which show selective degeneration of dopaminergic neurons, surviving neurons express only SUR1 subunits [56]. This suggests that KATP channels comprised of SUR1+Kir6.2 subunits enable neurons to survive severe metabolic inhibition. These data imply a neuroprotective role for neurons possessing a specific type of KATP channel. If hypothalamic GE neurons fell into this category, their maximal glucose sensitivity should be well below the levels required to initiate the counterregulatory response. We have shown that this is not the case [41]. This suggests that hypothalamic GE neurons possessing KATP channels differ from other KATP-channel-possessing neurons. If this is true, GE neurons may be particularly vulnerable to hypoxia and metabolic inhibition. There are no data as yet regarding this issue.

How then do GE neurons differ from other KATP-channel-containing neurons? Two hypotheses, which are not mutually exclusive, come to mind. First, there may be a difference in their KATP channel either in terms of subunit composition or regulation of ATP sensitivity or both. Support for the former derives from the fact that regions with glucose-sensing neurons (e.g., hypothalamus) have a relatively greater number of low affinity sulfonylurea binding sites (SUR2) and fewer high affinity binding sites (SUR1) than other regions of the brain [51]. This suggests differential expression of KATP channel subunits. Furthermore, the phosphorylation state of the KATP channel has an impact on its ATP sensitivity [57–59]. Since many neurotransmitters and neuromodulators exert their effects via phosphorylation, the local milieu could alter the ATP sensitivity of the KATP channel of GE neurons in the absence of differential subunit expression. A second hypothesis supporting a role for GE neurons in glucose homeostasis rather than neuronal protection derives from a further similarity to the pancreatic β -cell. Beta cells sense small changes in extracellular glucose with a special hexokinase known as glucokinase (GK, hexokinase IV) whose K_m is in the physiologic range for extracellular glucose [60]. GK is located in brain regions that contain glucose-sensing neurons [28,61–64]. Thus, while other KATP-channel-containing neurons contain hexokinase I, which is saturated at physiologic glucose levels [65–67], GE neurons may use GK to sense small changes in extracellular glucose. The data presented above provide compelling evidence that GE neurons differ from other KATP containing neurons. Thus, the primary role of hypothalamic glucose-sensing neurons may not be neuronal protection under such situations as hypoxia and stroke.

4.2. Glucose sensing (regulation of glucose homeostasis)

The fact that glucose-sensing neurons are capable of responding to physiologic changes in extracellular glucose (directly and via presynaptic input) makes it possible to suggest specific roles that they might play in the regulation of glucose homeostasis. But first, a discussion of the potential roles is needed. There are at least two possible functions for glucose-sensing neurons in the brain. These are initiation of the counterregulatory response and regulation of meal initiation and termination.

4.2.1. Initiation of the counterregulatory response

One possible function for glucose-sensing neurons involves sensing and initiating the counterregulatory response to hypoglycemia. When plasma glucose levels fall between 2 and 3 mM, sympathoadrenal activation occurs [2,3]. This increases plasma epinephrine, norepinephrine and glucagon, which, in turn, causes hepatic glycogenolysis and inhibition of pancreatic insulin secretion [14]. Sympathoadrenal activation is an extremely important protective mechanism, which increases glucose availability to the brain. Where this drop in glucose is sensed remains controversial. However, the brain appears plays an important role. Studies by Sherwin et al. [4,5,68] indicate that the VMN influences the secretion of the counterregulatory hormones epinephrine and glucagon. Rats with VMH lesions had markedly reduced secretion of epinephrine, norepinephrine and glucagon in response to insulin-induced hypoglycemia [4]. Moreover, when D-glucose, but not L-glucose, was delivered into the VMN the secretion of epinephrine, norepinephrine and glucagon in response to systemic hypoglycemia was significantly reduced [68].

Recent evidence indicates that the brainstem may also play an important role in sensing and initiating the counterregulatory response. Localized injection of the nonmetabolizable glucose analog, 2-deoxyglucose (2DG), into the brainstem catecholamine cell groups blocks local glucose utilization. This causes a type of hyperphagia known as glucoprivic feeding and increases plasma glucose [6]. Furthermore, the activity of these brainstem nuclei is altered during hypoglycemia associated autonomic failure (HAAF) [8]. HAAF, which occurs after only one bout of insulin-induced hypoglycemia or peripheral injection of 2DG, is associated with attenuated plasma epinephrine, norepinephrine and glucagon responses to a subsequent hypoglycemic challenge [69]. Hyperphagia and autonomic signs such as sweating are also diminished in HAAF. This is not permanent, and rats recover within 3–4 weeks of normoglycemia. In these studies, initial exposure to peripheral 2DG caused glucoprivic feeding and increased blood glucose as expected [8]. These responses were severely diminished upon reexposure to 2DG. Similarly, initial exposure to 2DG increased fos immunoreactivity (fos-ir; index of neuronal activation) in many brainstem regions, as well as in the hypothalamic paraventricular nucleus (PVN), but not in the VMN. After

the second exposure to 2DG, fos-ir was not induced in the brainstem sites and PVN [8]. Interestingly, selective destruction of brainstem epinephrine and norepinephrine neurons projecting to the PVN attenuate the glucocorticoid response and glucoprivic feeding but not the glucagon response to 2DG [70,71]. In contrast, destruction of spinally projecting catecholamine neurons impairs the glucagon response and hyperglycemia but not glucoprivic feeding in response to 2DG [72]. These data suggest that glucose-sensing neurons in brainstem catecholamine cell groups may be important in sensing and initiating the counterregulatory response to 2DG-induced glucoprivation. It is important to note that fos-ir is only a marker of neuronal activation and gives no information about neurons whose activity is decreased during hypoglycemia. This may explain the lack of response in the VMN. Beverly et al. [73] have shown that hypoglycemia results in GABA release in the VMN, and, thus, neurons in this region would be inhibited during glucoprivation.

Glucose-sensing neurons have also been described electrophysiologically in several brainstem nuclei, including the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus (DMV) [74–76]. However, these studies were performed using glucose levels outside the normal physiologic range. Thus, it is not certain that these brainstem glucose-sensing neurons could be involved in sensing and initiating counterregulation. However, in our studies of the VMN GE neurons, we found that their EC_{50} for glucose is 0.64 mM (unpublished observation). Therefore, they would be extremely sensitive to changes in extracellular glucose around the 0.2–0.5-mM range for extracellular brain glucose associated with plasma levels seen during counterregulation. Further support for a counterregulatory role for GE neurons comes from the work of Miki et al. [77] using Kir6.2 knockout mice. These mice lack functional KATP channels. Both glucoprivic feeding and glucagon secretion in response to hypoglycemia were attenuated in the Kir6.2 knockout mice. Interestingly, their feeding responses to NPY, and leptin were normal. These data suggest that the KATP channel (and presumably GE neurons) is important for sensing hypoglycemia and initiating glucoprivic feeding and glucagon release. However, KATP channels do not appear to be critical for normal feeding. Finally, the contribution of peripheral glucose sensors to counterregulation cannot be ignored. When vagal afferents from the portal vein are destroyed, epinephrine and norepinephrine secretion during hypoglycemia is severely attenuated [12].

4.2.2. Meal initiation and termination

A second role for glucose-sensing neurons is the regulation of meal initiation and termination. Considering the current obesity epidemic of most industrialized societies, understanding the regulation of this behavior is obviously critical. If glucose-sensing neurons were involved, this would provide an ideal drug target for treatment of obesity

and Type 2 diabetes mellitus. Normal plasma glucose levels during the day range between 80 and 120 mg/dl or about 5–8 mM. Early studies in rats indicate that meal initiation is preceded by drops in plasma glucose as small as 6–8% [78]. Campfield and Smith [79] later used on-line glucose monitoring to show that meal initiation actually occurred during a rise in plasma glucose preceded by a drop of about 10%. Little is known about the exact relationship between plasma glucose levels and meal initiation/termination. Studies showing that manipulation of plasma glucose within these ranges produces meal initiation are lacking. However, based on the data of Campfield and Smith [79], it is obvious that glucose-sensing neurons in the brain must be sensitive to extremely small changes in extracellular glucose. Assuming that brain extracellular glucose is about 1–2 mM under euglycemic conditions, a 10% drop would correspond to a change of approximately 100–200 μ M. This is a far cry from the 0–10-mM changes used to characterize these neurons in most studies. However, we have recently shown that GE neurons, at least, are sensitive to 100- μ M increments in extracellular glucose levels (unpublished observation). This would suggest that may be possible for glucose-sensing neurons to be involved in meal initiation and termination.

In order for glucose-sensing neurons to play a role in meal initiation and termination, they must exist in regions that are associated with food intake and energy balance. A number of investigators have shown this. These areas include the PVN [80], ARC [25,27,81], VMN [19,27,41] and NTS [74–76]. It is important to note that glucose-sensing neurons in brainstem regions may not be restricted to a role in counterregulation. Ferreria et al. found that glucose-sensing neurons in the NTS modulate gastric motility. These NTS neurons sensed glucose directly via the KATP channel and presynaptically modulate neurons in the DMV [76]. Thus, glucose-sensing neurons are sensitive to very small changes in extracellular glucose that may be involved in meal initiation, are anatomically well situated to regulate food intake and energy balance and modulate gastric motility.

In order to begin to suggest roles for specific subtypes of glucose-sensing neurons in the overall regulation of glucose homeostasis, we must turn to their sensitivity to changes in extracellular glucose within the physiologic range. We have found that the range of maximum sensitivity to glucose for the GE neurons is below 1 mM [41]. We have preliminary data indicating that the EC_{50} for glucose in GE neurons is 0.64 mM. According to the extracellular brain glucose levels determined by Silver and Erecinska [32], this places the maximum glucose sensitivity of these neurons within the range seen in the brain during initiation of the counterregulatory response to hypoglycemia. This would be consistent with the data of Miki et al. [77] showing that the glucagon response during counterregulation is attenuated in the absence of functional KATP channels. We have also determined that GI and PED neurons respond to a decrease

in extracellular glucose from 2.5 to 1 mM (Ref. [41] and unpublished observation). This places the glucose sensitivity of these neurons in the low range of brain levels during plasma euglycemia. Thus, they may regulate meal initiation. Finally, PER neurons and perhaps PIR neurons are sensitive to glucose changes between 2.5 and 5 mM [41]. Therefore, their glucose sensitivity is in the range the brain would see as plasma glucose levels transition from euglycemia to hyperglycemia. Thus, they may modulate either meal termination or the increased sympathetic activation seen during hyperglycemia. The key question mentioned earlier is what exactly are brain glucose levels. This will ultimately determine the role of these glucose-sensing neurons in glucose sensing. However, our work establishes that these neurons are functioning within the physiologic range. Moreover, they are capable of serving as glucose sensors involved in initiating counterregulation and/or meal initiation/termination rather than just serving a neuroprotective role when glucose levels fall to the point that brain function is compromised.

5. Are glucose-sensing neurons integrators of metabolic signals as well?

Regulation of energy balance entails more than just the regulation of plasma glucose. Energy balance involves energy intake, energy storage and energy utilization. All of these components are under the regulation of the autonomic nervous system [14,15]. As mentioned above, when plasma glucose levels fall to 2–3 mM, a sympathetically mediated increase in epinephrine release from the adrenal medulla occurs in order to increase liver glucose production and raise plasma glucose [2,3]. On the other hand, during hyperglycemia, sympathetic drive to skeletal muscle increases in order to increase peripheral glucose utilization and decrease plasma glucose [1]. Increased plasma glucose also increases insulin levels that promote glucose entry into tissues such as muscle and adipose; the latter promoting glucose storage as fat [14]. Thus, shifts in plasma glucose affect the energetic status of the body. In addition, glucose-sensing neurons are located in regions of the brain associated with energy balance [15]. They are also dysfunctional under conditions where energy balance is disturbed. The obese (*fa/fa*) Zucker rat has dysfunctional K^+ channels and GR neurons [25,82]. Moreover, we have demonstrated that all subtypes of physiologically relevant glucose-sensing neurons are both dysfunctional and fewer in number in rats that are prone to develop diet-induced obesity and insulin resistance [41]. This is associated with a 50% reduction in the sensitivity of the KATP channel to ATP and SUR [83].

These data suggest an important interaction between the regulation of glucose homeostasis and overall energy balance. Thus, it is likely that glucose-sensing neurons also act as metabolic integrators. A number of observations support this hypothesis. Spanswick et al. [25,27] have shown that VMN GR neurons are inhibited by both insulin and leptin

via activation of KATP channels. Oomura et al. [26,84] have shown that orexin, as well as low levels of leptin, increases the action potential frequency of medial hypothalamic GR neurons. In addition, GS neurons are responsive to leptin and orexin [24,26]. Moreover, ARC NPY neurons appear to be GS neurons [85] and ARC POMC to be GR neurons [86]. Finally, preliminary data from our lab shows that NE inhibits GE neurons (unpublished observation). Thus, glucose-sensing neurons respond to other important peptides and transmitters involved in the regulation of energy balance. It is apparent that these glucose-sensing neurons could serve as integrators of metabolic signals, as well as glucose, and be involved in overall energy homeostasis.

A second question related to the integrative function of glucose-sensing neurons is whether they specifically sense glucose or whether they respond to any form of energy substrate that produces ATP. Astrocytes sequester glucose and convert it to glycogen for storage. When energy levels drop, this glycogen is converted to lactate and transported out of astrocytes and into neurons via monocarboxylate acid transporters. Neurons convert the lactate into pyruvate using lactate dehydrogenase. Pyruvate then fuels mitochondrial oxidative phosphorylation [87]. It is not known if glucose-sensing neurons can use lactate as a signaling molecule similar to glucose. In order for glucose-sensing neurons to act as specific glucose sensors, they should be more sensitive to glucose than lactate under conditions when energy is not limited. The data regarding this are controversial. Yang et al. have shown that VMH glucose-sensing neurons, which increased their action potential frequency in response to increases in glucose over 5 mM, were similarly stimulated by early glycolytic intermediates. High levels of lactate (15 mM) also increased the action potential frequency of glucose-sensing neurons when given in the presence of 5 mM glucose [28]. This would suggest that these glucose-sensing neurons respond to any metabolic substrate that increases ATP levels. However, steady-state lactate levels in the brain normally range between 0.5 and 1.5 mM [87,88]. Additionally, 15 mM pyruvate did not alter neuronal activity in the presence of 5 mM glucose. Pyruvate did, however, increase action potential frequency when glucose levels were 0 mM [28]. Our preliminary data suggest that 0.5 mM lactate can substitute for glucose in both GE and GI neurons when energy levels are very low (0.1 mM glucose) [89]. We do not know if the same will occur when glucose is not limiting. Thus, it is important to carefully address these issues using physiologic levels of both glucose and lactate.

In conclusion, glucose-sensing neurons respond to physiologic changes in extracellular glucose. The evidence described above supports the hypothesis that they play a role in the regulation of glucose homeostasis. A comparison of the respective glucose sensitivities of the subtypes of hypothalamic glucose-sensing neurons has led us to tentatively hypothesize specific roles in the regulation of glucose homeostasis. GE neurons may be involved in sensing and initiating the counterregulatory response to hypoglycemia.

On the other hand, four other subtypes of VMN glucose-sensing neurons respond to changes in extracellular glucose, which may occur normally throughout the day. Thus, we postulate that these glucose-sensing neurons (GI, PED, PER and PIR) may play a role in meal initiation and termination. Finally, it is also likely that glucose-sensing neurons are not merely glucose sensors. Rather, they function as metabolic integrators to enable the brain to maintain overall energy homeostasis.

References

- [1] Hoffman RP, Hausberg M, Sinkey CA, Anderson EA. Hyperglycemia without hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Diabetes Comp* 1999; 13:17–22.
- [2] Amiel SA, Tamborlane WV, Simonson DC, Sherwin RS. Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. *N Eng J Med* 1987;316:1376–83.
- [3] Cryer PE. Glucose counterregulation in man. *Diabetes* 1981;30: 261–4.
- [4] Borg WP, During MJ, Sherwin RS, Borg MA, Brines ML, Shulman GI. Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. *J Clin Invest* 1994;93:1677–82.
- [5] Borg WP, Sherwin RS, During MJ, Borg MA, Shulman GI. Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. *Diabetes* 1995;44:180–4.
- [6] Ritter S, Dinh TT, Zhang Y. Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain Res* 2000; 856:37–47.
- [7] Ritter S, Llewellyn-Smith I, Dinh TT. Subgroups of hindbrain catecholamine neurons are selectively activated by 2-deoxy-D-glucose induced metabolic challenge. *Brain Res* 1998;805:41–54.
- [8] Sanders NM, Ritter S. Repeated 2-deoxy-D-glucose-induced glucoprivation attenuates Fos expression and glucoregulatory responses during subsequent glucoprivation. *Diabetes* 2000;49:1865–74.
- [9] Nijijima A. Glucose-sensitive afferent nerve fibres in the hepatic branch of the vagus nerve in the guinea-pig. *J Physiol* 1982;332:315–23.
- [10] Shimizu N, Oomura Y, Novin D, Grijalva CV, Cooper PH. Functional correlations between lateral hypothalamic glucose-sensitive neurons and hepatic portal glucose-sensitive units in rat. *Brain Res* 1983; 265:49–54.
- [11] Hevener AL, Bergman RN, Donovan CM. Hypoglycemic detection does not occur in the hepatic artery or liver: findings consistent with a portal vein glucosensor locus. *Diabetes* 2001;50:399–403.
- [12] Hevener AL, Bergman RN, Donovan CM. Portal afferents are critical for the sympathoadrenal response to hypoglycemia. *Diabetes* 2000; 49(1):8–12.
- [13] Alvarez-Buylla R, de Alvarez-Buylla ER. Carotid sinus receptors participate in glucose homeostasis. *Respir Physiol* 1998;72:347–60.
- [14] Nonogaki K. New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia* 2000;43:533–49.
- [15] Levin BE, Dunn-Meynell AA, Routh VH. Brain glucose sensing and body energy homeostasis: role in obesity and diabetes. *Am J Physiol* 1999;276:R1223–31.
- [16] Anand BK, China GS, Sharma KN, Dua S, Singh B. Activity of single neurons in the hypothalamus feeding centers: effect of glucose. *Am J Physiol* 1964;2207:1146–54.
- [17] Oomura Y, Kimura K, Ooyama H, Maeo T, Iki M, Kuniyoshi N. Reciprocal activities of the ventromedial and lateral hypothalamic area of cats. *Science* 1964;143:484–5.
- [18] Oomura Y, Ono H, Ooyama H, Wayner MJ. Glucose and osmosensitive neurons of the rat hypothalamus. *Nature* 1969;222:282–4.
- [19] Ashford MLJ, Boden PR, Treherne JM. Tolbutamide excites rat glucoreceptive ventromedial hypothalamic neurones by indirect inhibition of ATP-sensitive K⁺ channels. *Br J Pharmacol* 1990;101: 531–40.
- [20] Ashcroft FM, Gribble FM. ATP-sensitive K⁺ channels and insulin secretion: their role in health and disease. *Diabetologia* 1999;42: 903–19.
- [21] Silver IA, Erecinska M. Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. *J Neurophysiol* 1998;79: 1733–45.
- [22] Oomura Y. Glucose as a regulator of neuronal activity. *Adv Metab Disord* 1983;10:31–65.
- [23] Ashford MLJ, Boden PR, Treherne JM. Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K⁺ channels. *Eur J Physiol* 1990;415:479–83.
- [24] Funahashi H, Yada T, Muroya S, Takigawa M, Ryushi T, Horie S, Nakai Y, Shioda S. The effect of leptin on feeding-regulating neurons in the rat hypothalamus. *Neurosci Lett* 1999;264:117–20.
- [25] Spanswick D, Smith MA, Mirshamsi S, Routh VH, Ashford MLJ. Insulin activates ATP-sensitive K⁺ channels in hypothalamic neurons of lean, but not obese rats. *Nat Neurosci* 2000;3:757–8.
- [26] Shiraishi T, Oomura Y, Sasaki K, Wayner MJ. Effects of leptin and orexin-A on food intake and feeding related hypothalamic neurons. *Physiol Behav* 2000;71:251–61.
- [27] Spanswick D, Smith MA, Groppi VE, Logan SD, Ashford MLJ. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature* 1997;390:521–5.
- [28] Yang XJ, Kow LM, Funabashi T, Mobbs CV. Hypothalamic glucose sensor: similarities to and differences from pancreatic beta-cell mechanisms. *Diabetes* 1999;48:1763–72.
- [29] McNay EC, Fries TM, Gold PE. Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. *PNAS* 2000;97:2881–5.
- [30] McNay EC, Gold PE. Extracellular glucose concentrations in the rat hippocampus measured by zero-net-flux: effects of microdialysis flow rate, strain, and age. *J Neurochem* 1999;72:785–90.
- [31] McNay EC, McCarty RC, Gold PE. Fluctuations in brain glucose concentration during behavioral testing: dissociations between brain areas and between brain and blood. *Neurobiol Learn Mem* 2001;75: 325–37.
- [32] Silver IA, Erecinska M. Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. *J Neurosci* 1994;14:5068–76.
- [33] Ross BD, Bluml S, Cowan R, Danielsen E, Farrow N, Gruetter R. In vivo magnetic resonance spectroscopy of human brain: the biophysical basis of dementia. *Biophys Chem* 1997;68:161–72.
- [34] Gruetter R, Garwood M, Uğurbil K, Seaquist ER. Observation of resolved glucose signals in 1H NMR spectra of the human brain at 4 Tesla. *Magn Reson Med* 1996;36:1–6.
- [35] Fellows LK, Boutelle MG, Fillenz M. Extracellular brain glucose levels reflect local neuronal activity: a microdialysis study in awake, freely moving rats. *J Neurochem* 1992;59:2141–7.
- [36] Fray AE, Boutelle MG, Fillenz M. Extracellular glucose turnover in the striatum of unanaesthetized rats measured by quantitative microdialysis. *J Physiol* 1997;504:721–7.
- [37] Ahima RS, Saper CB, Flier JS, Elmquist JK. Leptin regulation of neuroendocrine systems. *Front Neuroendocrinol* 2000;21:263–307.
- [38] Hu Y, Wilson GS. Rapid changes in local extracellular glucose observed with an in vivo glucose sensor. *J Neurochem* 1997;68:1745–52.
- [39] Netchiporouk LI, Shram NF, Jaffrezic-Renault N, Martelet C, Caspoglio R. In vivo brain glucose measurements: differential normal pulse voltammetry with enzyme-modified carbon fiber microelectrodes. *Anal Chem* 1996;68:4358–64.
- [40] Ronne-Engstrom E, Carlson H, Yansheng L, Ungerstedt UHL. Influence of perfusate glucose concentration on dialysate lactate, pyruvate, aspartate, and glutamate levels under basal and hypoxic conditions: a microdialysis study in rat brain. *J Neurochem* 1995;65:257–62.

- [41] Song Z, Levin BE, McArdle JJ, Bakhos N, Routh VH. Convergence of pre- and postsynaptic influences on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes* 2001;50:2673–81.
- [42] Baukrowitz T, Hwang T-C, Nairn AC, Gadsby DC. Coupling of CFTR Cl⁻ channel gating to an ATP hydrolysis cycle. *Neuron* 1994; 12:473–82.
- [43] Hwang T-C, Sheppard DN. Molecular pharmacology of the CFTR Cl⁻ channel. *Trends Pharmacol Sci* 1999;20:448–53.
- [44] Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 1997;278:135–8.
- [45] Weyler RT, Yurko-Mauro KA, Rubenstein R, Kollen WJ, Reenstra W, Altschuler SM, Egan M, Mulberg AE. CFTR is functionally active in GnRH-expressing GT1-7 hypothalamic neurons. *Am J Physiol* 1999; 277:C563–71.
- [46] Mulberg AE, Weidner EB, Bao X, Marshall J, Jefferson DM, Altschuler SM. Cystic fibrosis transmembrane conductance regulator protein expression in brain. *NeuroReport* 1994;5:1684–8.
- [47] Mulberg AE, Weyler RT, Altschuler SM, Hyde TM. Cystic fibrosis transmembrane conductance regulator expression in human hypothalamus. *NeuroReport* 1998;9:141–4.
- [48] Levin BE. Glucose-regulated dopamine release from substantia nigra neurons. *Brain Res* 2000;874:158–64.
- [49] During MJ, Leone P, Davis KE, Kerr D, Sherwin RS. Glucose modulates rat substantia nigra GABA release in vivo via ATP-sensitive potassium channels. *J Clin Invest* 1995;95:2403–8.
- [50] Aguilar-Bryan L, Clement JP, Gonzalez G, Kunjilwar K, Babenko A, Bryan J. Toward understanding the assembly and structure of K-ATP channels. *Physiol Rev* 1998;78:227–45.
- [51] Dunn-Meynell AA, Routh VH, McArdle JJ, Levin BE. Low-affinity sulfonylurea binding sites reside on neuronal cell bodies in the brain. *Brain Res* 1997;745:1–9.
- [52] Chutkow WA, Makielski JC, Nelson DJ, Burant CF, Fan Z. Alternative splicing of sur2 Exon 17 regulates nucleotide sensitivity of the ATP-sensitive potassium channel. *J Biol Chem* 1999;274:13656–65.
- [53] Liss B, Bruns R, Roeper J. Alternative sulfonylurea receptor expression defines metabolic sensitivity of K-ATP channels in dopaminergic midbrain neurons. *EMBO J* 1999;18:833–46.
- [54] Zini S, Roisin MP, Armengaud C, BenAri Y. Effect of potassium channel modulators on the release of glutamate induced by ischemic conditions in rat hippocampal slices. *Neurosci Lett* 1993; 153(2):202–5.
- [55] Dunn-Meynell AA, Rawson NE, Levin BE. Distribution and phenotype of neurons containing the ATP-sensitive K⁺ channel in rat brain. *Brain Res* 1998;814:41–54.
- [56] Liss B, Neu A, Roeper J. The *weaver* mouse *gain-of-function* phenotype of dopaminergic midbrain neurons is determined by coactivation of *wvGirk2* and K-ATP channels. *J Neurosci* 1999;19:8839–48.
- [57] Routh VH, McArdle JJ, Levin BE. Phosphorylation modulates the activity of the ATP-sensitive K⁺ channel in the ventromedial hypothalamic nucleus. *Brain Res* 1997;778:107–19.
- [58] Baukrowitz T, Schulte U, Oliver D, Herlitz S, Krauter T, Tucker SJ, Ruppertsberg JP, Fakler B. PIP2 and PIP as determinants for ATP inhibition of KATP channels. *Science* 1998;282:1141–4.
- [59] Shyng S-L, Nichols CG. Membrane phospholipid control of nucleotide sensitivity of KATP channels. *Science* 1998;282:1138–41.
- [60] Matschinsky FM, Glaser B, Magnuson MA. Pancreatic beta-cell glucokinase: closing the gap between theoretical concepts and experimental realities. *Diabetes* 1998;47:307–15.
- [61] Roncero I, Alvarez E, Vazquez P, Blazquez E. Functional glucokinase isoforms are expressed in rat brain. *J Neurochem* 2000;74: 1848–57.
- [62] Jetton TL, Liang Y, Pettepher CC, Zimmerman EC, Cox FG, Horvath FM, Matschinsky FM, Magnuson MA. Analysis of upstream glucokinase promoter activity in transgenic mice and identification of glucokinase in rare neuroendocrine cells in the brain and gut. *J Biol Chem* 1994;269:3641–54.
- [63] Navarro M, Rodriguez DF, Alvarez E, Chowen JA, Zueco JA, Gomez R, Eng J, Blazquez E. Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *J Neurochem* 1996;67:1982–91.
- [64] Lynch RM, Tompkins LS, Brooks HL, Dunn-Meynell AA, Levin BE. Localization of glucokinase gene expression in the rat brain. *Diabetes* 2000;49:693–700.
- [65] Liu F, Dong Q, Myers AM, Fromm HJ. Expression of human brain hexokinase in *Escherichia coli*: purification and characterization of the expressed enzyme. *Biochem Biophys Res Commun* 1991;177: 305–11.
- [66] Magnuson MA. Glucokinase gene structure. Functional implications of molecular genetic studies. *Diabetes* 1990;39:523–7.
- [67] Garner JA, Linse KD, Polk RK. Type I brain hexokinase: axonal transport and membrane associations within central nervous system presynaptic terminals. *J Neurochem* 1996;67:845–56.
- [68] Borg MA, Sherwin RS, Borg WP, Tamborlane WV, Shulman GI. Local ventromedial hypothalamus glucose perfusion blocks counter-regulation during systemic hypoglycemia in awake rats. *J Clin Invest* 1997;99:361–5.
- [69] Heller SR, Cryer PE. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes* 1991;40:223–6.
- [70] Ritter S, Dinh TT, Sanders NM, Pedrow C. Selective immunotoxin lesion of hypothalamically-projecting norepinephrine/epinephrine (Ne/E) neurons impairs the glucocorticoid response to glucoprivation. *Abstr Soc Neurosci* 2001;31:947.
- [71] Sanders NM, Dinh TT, Pedrow C, Ritter S. Selective immunotoxin lesions of hindbrain norepinephrine/epinephrine (Ne/E) neurons impair feeding and corticosterone responses and fos-immunoreactivity in hypothalamic sites during insulin-induced hypoglycemia. *Abstr Soc Neurosci* 2001;31:635.
- [72] Dinh TT, Sanders NM, Pedrow C, Ritter S. Selective immunotoxin lesion of spinally projecting norepinephrine and epinephrine (Ne/E) neurons impairs the glucagon response to 2-deoxy-D-glucose (2DG). *Abstr Soc Neurosci* 2001;31:947.
- [73] Beverly JL, De Vries MG, Beverly MF, Arseneau LM. Norepinephrine mediates glucoprivic-induced increase in GABA in the ventromedial hypothalamus of rats. *Am J Physiol* 2000;279:R990–6.
- [74] Mizuno Y, Oomura Y. Glucose responding neurons in the nucleus tractus solitarius of the rat, in vitro study. *Brain Res* 1984;307: 109–16.
- [75] Dallaporta M, Perrin J, Orsini JC. Involvement of adenosine triphosphate-sensitive K⁺ channels in glucose-sensing in the rat solitary tract nucleus. *Neurosci Lett* 2000;278:77–80.
- [76] Ferreira M. Glucose effects on gastric motility and tone evoked from the rat dorsal vagal complex. *J Physiol* 2001;536:141–52.
- [77] Miki T, Liss B, Minami K, Shiuchi T, Saraya A, Kashima Y, Horiuchi M, Ashcroft FM, Minokoshi Y, Roeper J, Seino S. ATP-sensitive potassium channels in hypothalamic neurons play an essential role in the maintenance of glucose homeostasis by controlling glucagon release and food intake. *Nat Neurosci* 2001;5:507–12.
- [78] Louis-Sylvestre J, Le Magnen J. A fall in blood glucose precedes meal onset in free-feeding rats. *Neurosci Biobehav Rev* 1980;4(Suppl 1): 13–15.
- [79] Campfield LA, Smith FJ. Functional coupling between transient declines in blood glucose and feeding behavior: temporal relationships. *Brain Res Bull* 1986;17:427–549.
- [80] Kow LM, Pfaff DW. Responses of hypothalamic paraventricular neurons in vitro to norepinephrine and other feeding-relevant agents. *Physiol Behav* 1989;46:265–71.
- [81] Wang R, Ingoglia NA, Routh VH. Arcuate (ARC) glucosensing neurons respond to physiologic changes in extracellular glucose. *Abstr Soc Neurosci* 2001;31:947.
- [82] Rowe IC, Boden PR, Ashford MLJ. Potassium channel dysfunction in

- hypothalamic glucose-receptive neurones of obese Zucker rats. *J Physiol* 1996;497:365–77.
- [83] Routh VH, Levin BE, McArdle JJ. Defective ATP and sulfonylurea sensitivity of the ATP-sensitive K^+ (KATP) channel in the ventromedial hypothalamic nucleus (VMN) of obesity-prone (DIO) rats. *FASEB J* 2001;12:A864.
- [84] Shiraishi T, Sasaki K, Nijima A, Oomura Y. Leptin effects on feeding-related hypothalamic and peripheral neuronal activities in normal and obese rats. *Nutrition* 1999;15:576–9.
- [85] Muroya S, Yada T, Shioda S, Takigawa M. Glucose-sensitive neurons in the rat arcuate nucleus contain neuropeptide Y. *Neurosci Lett* 1999;264:113–6.
- [86] Ibrahim N, Smart JL, Rubenstein R, Low MJ, Kelly M. Mouse hypothalamic POMC neurons are modulated by K_{ATP} channel activity. *Abstr Soc Neurosci* 2001;31:733.
- [87] Ames AI. CNS energy metabolism as related to function. *Brain Res Rev* 2000;34:42–68.
- [88] Bliss TM, Sapolsky RM. Interactions among glucose, lactate and adenosine regulate energy substrate utilization in hippocampal cultures. *Brain Res* 2001;899:134–41.
- [89] Song Z, Routh VH. Differential effects of lactate on glucosensing neurons in the ventromedial hypothalamic nucleus (VMN). *Soc Neurosci Abstr* 2001;947.2.