Glucokinase activity in the arcuate nucleus regulates glucose intake

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Introduction

It has been suggested that glucose, the brain’s primary fuel source, regulates food intake (1). Although glucose injections into the CNS reduce food intake, glucose is preferred to other types of food by rodents and humans (2–4). Therefore, a mechanism to detect glucose in food and promote the intake of glucose-rich foods is likely to exist. The regulation of glucose intake has been studied extensively (5, 6). A system regulating glucose intake, driven by hedonic responses generated in the limbic system, has been identified (7). Dopamine is thought to be important in the hedonic response to glucose (8). Evidence suggests that nonhedonic nontaste systems are important in regulating glucose intake (9) and that metabolism of glucose is an important factor (10). However, a nontaste-dependent homeostatic mechanism within the hypothalamus or elsewhere in the brain has proved elusive.

Glucokinase is a member of the hexokinase family of enzymes, which phosphorylates glucose to form glucose-6 phosphate (11). Glucokinase is expressed in the liver, pancreas, and CNS (11, 12). Glucokinase is expressed in 2 isoforms: a hepatic form expressed in the liver and a neuroendocrine form expressed in the pancreas and CNS (13). The 2 isoforms are produced by the utilization of different promoters. The isoforms have the same kinetic properties but different functions (13). Within the β cells and glucose-sensi-

tive neurons glucokinase is part of a glucose-sensing system (14). The mechanism used for the glucose-sensing system is thought to be similar in both β cells and neurons (14). Glucose entry into the cell is via the GLUT-2 transporter (15). Metabolism of glucose by glucokinase results in closure of the ATP-sensitive potassium (K\text{ATP}) channels. This results in depolarization of the cell and release of hormone via a calcium-dependent mechanism.

In the pancreas and liver, glucokinase has an important role in regulating glucose homeostasis (11). Its role in the CNS is less clear. In the hypothalamus, glucokinase is expressed in regions, including the arcuate nucleus, ventromedial nucleus (VMN), paraventricular nucleus (PVN), and lateral hypothalamic area (LHA), which regulate energy homeostasis and are part of a CNS glucose-sensing system (14, 16, 17). In the VMN, glucokinase is involved in mediating the hormonal counterregulatory responses to hypoglycemia and does not regulate energy homeostasis (18, 19). While a role for hypothalamic glucokinase in regulating energy homeostasis has been proposed, it has never been confirmed (12, 14). We hypothesized that glucokinase in the hypothalamic arcuate nucleus was involved in the regulation of energy homeostasis. We found that increased arcuate nucleus glucokinase activity increased food intake and weight gain in rats. It also increased glucose intake in preference to other food types. These effects were mimicked by intra-arcuate administration of glibenclamide, which blocks the K\text{ATP} channel. Converse effects were obtained by reducing glucokinase activity in the arcuate nucleus and with intra-arcuate injection of diazoxide, a K\text{ATP} channel activator. We identified a likely mechanism involving P/Q voltage-gated calcium channels, NPY release, and action
To determine the long-term effect of increased glucokinase in the arcuate nucleus, we used recombinant adeno-associated virus (rAAV) to overexpress the neuroendocrine isoform of glucokinase (rAAV-GK) in this nucleus. The enzymatic activity of the construct was tested in vitro in HEK293 cells. Cells transfected with rAAV-GK had significantly increased glucokinase activity compared with cells transfected with the control virus expressing GFP (rAAV-GFP) (Figure 1C). We stereotactically injected rAAV-GK into the arcuate nucleus of adult male Wistar rats (referred to herein as iARC-GK rats) and rAAV-GFP into the arcuate nucleus of control rats (referred to herein as iARC-GFP rats). In keeping with other studies using similar protocols (22), glucokinase activity was increased specifically in the arcuate nucleus of iARC-GK rats by 2.03 ± 0.30–fold compared with that in controls (Figure 1D). Glucokinase activity was not altered in either the VMN or the PVN (Figure 1D). Consistent with this, increased glucokinase expression was specifically localized to the hypothalamic arcuate nucleus in iARC-GK rats (Supplemental Figure 2, A and B). Expression of GFP, as detected by immunocytochemistry, was also limited to the arcuate nucleus in iARC-GFP animals (Supplemental Figure 2C). These results confirm the validity of iARC-GK rat as a model of increased arcuate nucleus glucokinase activity.

Compared with controls, iARC-GK rats had increased cumulative food intake, gained more weight, and had increased body fat when fed a standard chow diet (at 33 days, cumulative food intake: iARC-GFP, 1,094.3 ± 22.39 g; iARC-GK, 1,194.1 ± 24.92 g; iARC-GK rats had increased cumulative food intake, gained more weight, and had increased body fat when fed a standard chow diet (at 33 days, cumulative food intake: iARC-GFP, 1,094.3 ± 22.39 g; iARC-GK, 1,194.1 ± 24.92 g;
Increased glucokinase activity in the arcuate nucleus increases glucose intake in preference to chow. We hypothesized that arcuate nucleus glucokinase selectively stimulates intake of glucose-rich foods in preference to other foods. To investigate whether increased glucokinase activity in the arcuate nucleus increased glucose intake in preference to chow, we measured the intake of glucose and chow when ad libitum access to both was available. In this setting, injection of a glucokinase activator into the arcuate nucleus resulted in a 2.52 ± 0.51–fold increase in 2% w/v glucose intake in the first hour after injection (Figure 2D), which is consistent with our previous finding (Figure 2A). In contrast to when chow was presented alone (Figure 1B), CpdA did not increase chow intake when it was presented alongside glucose (vehicle, 1.69 ± 0.43 g; CpdA, 1.43 ± 0.70 g) (Figure 2E).

Similarly, in iARC-GK rats, 24-hour intake of 2%, 10%, and 20% w/v glucose was significantly increased (Figure 2F and Supplemental Figure 4, A and D). Twenty-four hour chow intake and total energy intake (derived from both chow and glucose) were not different between the 2 groups when glucose was available in addition to chow (Figure 2, G and H, and Supplemental Figure 4, B, C, E, and F). When chow was presented alone, chow intake cumulative weight gain: iARC-GFP, 151.67 ± 8.86 g; iARC-GK, 175.33 ± 4.87 g; body fat: iARC-GFP, 22.12% ± 1.73%; iARC-GK, 28.0% ± 1.62% (Figure 1, E and F, and Supplemental Figure 3A). Lean mass, brown adipose tissue (BAT) weight, BAT Ucp1 mRNA expression, and glucose and insulin levels were unaltered (Supplemental Figure 3, B–H). Two-hour food intake following a 48-hour fast was no different between the 2 groups (Supplemental Figure 3I). These data suggest that increased arcuate nucleus glucokinase activity increases food intake and bodyweight and support a role for arcuate nucleus glucokinase in the control of energy homeostasis.

Increased glucokinase activity in the arcuate nucleus increases glucose intake but not fructose intake. Since glucokinase is part of the glucose-sensing mechanism, we tested whether increased arcuate nucleus glucokinase activity affected the intake of glucose or fructose, a closely related sugar, which is not a substrate for glucokinase (23). Both pharmacologic and genetic activation of arcuate nucleus glucokinase increased intake of 2% and 10% w/v glucose solution (Figure 2, A–C). In contrast, intake of fructose solution was not affected (Figure 2, B and C). These data suggest that, in terms of sugar intake, arcuate nucleus glucokinase activity may specifically regulate glucose intake.
Decreased glucokinase activity in the arcuate nucleus decreases food intake and glucose intake. To examine the physiological role of arcuate glucokinase in the regulation of energy homeostasis and glucose consumption, we used rAAV encoding antisense glucokinase (rAAV-ASGK), which has previously shown to be effective at reducing glucokinase activity in vivo (24). The activity of the construct was tested in vitro in Hep g2 cells. Cells transfected with rAAV-ASGK had markedly reduced glucokinase activity compared with that of cells transfected with rAAV-GFP (Figure 3A).

We stereotactically injected rAAV-ASGK into the arcuate nucleus of adult male Wistar rats (referred to herein as iARC-ASGK rats); iARC-GFP rats were used as the control. Glucokinase activity was decreased specifically in the arcuate nucleus of iARC-GK rats to approximately 50% of that of control iARC-GFP rats (Figure 3B). Glucokinase activity was not altered in the VMN or the PVN (Figure 3B).

iARC-ASGK rats consumed less food (at 33 days, food intake: iARC-GFP, 1,039 ± 17.9 g; iARC-ASGK, 994 ± 14.1 g) (Figure 3C), and they also gained less weight, although this did not reach statistical significance (iARC-GFP, 153.9 ± 6.0 g; iARC-ASGK, 139 ± 6.2 g) (P = 0.06), when fed a standard chow diet compared with controls (Figure 3D). We tested the effect of reduced glucok

and therefore energy intake were increased in iARC-GK rats compared with controls (iARC-GFP, 25.86 ± 1.01 g; iARC-GK, 29.46 ± 1.27 g) (Figure 2I). This suggests that the increase in chow intake produced by glucokinase activation was driven by an increased appetite for glucose.

To determine whether this effect was sustained long term, iARC-GK and iARC-GFP rats were given ad libitum access to 10% w/v glucose and standard chow. Increased arcuate nucleus glucokinase activity resulted in increased glucose intake (at 31 days, iARC-GFP, 2,812.9 ± 91.89 ml; iARC-GK, 3,337.1 ± 131.39 ml) (Figure 2J). The previously observed increase in food intake, energy intake, and body weight gain in iARC-GK rats that occurred with chow alone did not occur when a glucose solution was available in addition to the chow diet (at 31 days, food intake: iARC-GFP, 691.94 ± 19.41 g; iARC-GK, 682.14 ± 22.84 g; energy intake: iARC-GFP, 14.41 ± 0.33 MJ; iARC-GK, 15.07 ± 0.42 MJ; weight gain: 61.00 ± 4.17 g; iARC-GK, 66.25 ± 6.12 g) (Figure 2, K–M). These data support a role for arcuate glucokinase in selectively regulating glucose intake and support our hypothesis that the orexigenic effect of increasing arcuate nucleus glucokinase activity is the result of an increased drive to consume glucose.

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We therefore hypothesized that inhibition of K\textsubscript{ATP} channels may be acting via inhibiting the K\textsubscript{ATP} channels, either the K\textsubscript{ATP} channel inhibitor, glibenclamide, or the K\textsubscript{ATP} channel activator, diazoxide, was injected into the arcuate nucleus of Wistar rats. Four hours after injection of glibenclamide, food intake was increased compared with that after control injections when chow was available alone (2.22 ± 0.77 relative to control) (Figure 4A). Similarly, 2% w/v glucose intake (2.73 ± 0.99 relative to control) was significantly increased 4 hours after glibenclamide injection compared with that after control injections when glucose was presented alone (Figure 4B). When both chow diet and glucose were present, only glucose intake was increased following glibenclamide injection, compared with that after control injection (1.93 ± 0.57 relative to control), with chow intake unaffected (0.97 ± 0.19 relative to control) (Figure 4C and D).

Conversely, injection of diazoxide into the arcuate nucleus inhibited food intake when chow was presented alone compared with that after control injections (Figure 4E and Supplemental Figure 5A). Glucose intake was also reduced following injection of diazoxide compared with that after control injections (Figure 4F and Supplemental Figure 5B). However, when both glucose and chow were available, only the consumption of glucose was decreased following diazoxide injection, whereas that of chow remained unchanged (Figure 4G and H, and Supplemental Figure 5, C and D).

The role of K\textsubscript{ATP} channels in mediating the increased glucose intake stimulated by glucokinase activation in the arcuate nucleus was investigated by coadministration of diazoxide and CpdA into the arcuate nucleus in fed animals. As before, injection of CpdA increased intake of glucose, and this increased glucose intake was blocked by coadministration of diazoxide (Figure 4I). Under the conditions used, diazoxide given alone did not affect glucose intake when administered alone (Figure 4I). These data are in accord with K\textsubscript{ATP} channels having a role in transducing the effects of arcuate nucleus glucokinase on glucose appetite.

Effects of arcuate nucleus glucokinase on hypothalamic AMPK, ACC, and FAS. We investigated possible mechanisms by which increased arcuate nucleus glucokinase activity may be exerting its effects on food and glucose intake. Increasing arcuate...
nucleus glucokinase activity did not affect either expression or activity of hypothalamic adenosine monophosphate–activated protein kinase (AMPK), a key cellular energy sensor that is influenced by cellular ATP levels (ref. 25 and Figure 5, A and B). In addition, there was no difference in expression of the metabolic sensing enzyme acetyl CoA carboxylase in iARC-GK animals compared with that in controls (ref. 26 and Figure 5A). There was a small increase in fatty acid synthase expression in iARC-GK animals compared with that in controls (Figure 5A). This enzyme catalyzes the synthesis of long-chain fatty acids and modulates food intake (27).

The effects of glucokinase on glucose intake are dependent upon calcium channel activity. To investigate whether the effects of arcuate nucleus glucokinase activity were dependent upon calcium channel activity specific calcium channel blockers were used. Either nifedipine, specific for L-type calcium channels, or ω-agonist IVA, specific for P/Q-type channels, was injected into the arcuate nucleus, followed by injection of CpdA and measurement of intake of 2% w/v glucose. Injection of nifedipine alone did not alter glucose intake at 1 hour (Figure 5C) or at subsequent time points (Supplemental Figure 6A), and it did not have any effect on glucose intake stimulated by CpdA at either 1 hour (Figure 5C)
The effects of altered glucokinase activity on NPY release. Release of NPY from ex vivo hypothalamic slices in response to increasing glucose concentrations was altered in iARC-GK animals compared with that in iARC-GFP controls. Treatment of hypothalamic slices from iARC-GFP rats with increasing concentrations of glucose resulted in a decrease in NPY release (Figure 5D). In contrast, treatment of hypothalamic slices from iARC-GK rats with increasing concentrations of glucose did not affect NPY release. Release of α-melanocyte-stimulating hormone (α-MSH) from ex vivo hypothalamic slices from either iARC-GFP or iARC-GK rats was not affected by changes in glucose concentration (Figure 5E).

A similar response was seen when hypothalamic slices were treated with either CpdA, a glucokinase activator, or glibenclamide, which blocks $K_{ATP}$ channels. Treatment with either of these 2 pharmacologic agents increased NPY release from ex vivo hypothalamic slices (Figure 5F). In contrast, treatment of ex vivo hypothalamic slices with diazoxide, which activates $K_{ATP}$ channels, reduced NPY release (Figure 5F). Release of α-MSH from hypothalamic slices was not altered by any treatment (Figure 5G). The increased release of NPY from the hypothalamic slices stimulated by glucokinase activation appears to be dependent on closure of the $K_{ATP}$ channel, since treatment of ex vivo slices with diazoxide blocked the effect of CpdA (Figure 5H). These data indicate that the effects of arcuate nucleus glucokinase on energy homeostasis may be mediated by the orexigenic neurotransmitter NPY.

**Discussion**

It has been suggested that glucose sensors, such as glucokinase, have a role in the hypothalamic regulation of food intake (1, 14). Increased glucokinase activity has been postulated but not demonstrated to reduce food intake as part of a glucostatic control system. We found that glucokinase activity increased in the arcuate nucleus following a fast. Expression of glucokinase is regulated by fasting in a tissue-specific manner. Expression of hepatic glucokinase is reduced by fasting (28), while expression of the neuroendocrine form of glucokinase in the pancreas is unaltered by fasting (28). In contrast, expression of the neuroendocrine form of glucokinase in the hypothalamus is increased by fasting (29). The mechanism underlying the differences in the regulation of expression is unclear; however, it is likely to be the result of differences in the promoter sequence used and the combination of transcription factors expressed in each tissue. In addition to this, regulation of glu-
Glucokinase occurs at a transcriptional level. It has been demonstrated recently that glucokinase activity in the hypothalamus is regulated by glucokinase regulatory protein (GKRP) (30). GKRP regulates glucokinase activity by binding to it and inactivating it by sequestering it within the nucleus (31). In the liver, the binding of GKRP to glucokinase is increased by hypoglycemia and fasting, resulting in reduced glucokinase activity, which is reduced in response to these stimuli. However, in the hypothalamus, binding of GKRP to glucokinase is inhibited by fasting and increased by hyperglycemia (30). Therefore, glucokinase activity in the hypothalamus would be increased with fasting, in line with our findings.

Injection of the glucokinase activator, CpdA, into the arcuate nucleus increased food intake. This is opposite from the effect proposed by the glucostatic hypothesis of food intake, although it is consistent with the increase in glucokinase activity detected following a fast. Many orexigenic agents demonstrate increased activity or expression in response to fasting, whereas anorexigenic agents demonstrate the opposite response (20). To investigate whether this effect was sustained, rAAV-GK was stereotactically injected into the arcuate nucleus of adult male iARC-GK rats. On a chow diet, iARC-GK animals consumed more food than iARC-GFP animals, in accord with the findings following pharmacologic activation. To investigate the physiological importance of this, we reduced arcuate nucleus glucokinase activity by injecting rAAV-ASGK into the arcuate nucleus of iARC-ASGK rats. On a chow diet, iARC-ASGK animals consumed less food than iARC-GFP animals. Together, these data suggest that increased arcuate nucleus glucokinase activity may constitute an orexigenic signal. This is consistent with previous work revealing increased hypothalamic glucokinase mRNA expression in diet-induced obese and obese-prone rats (32). Previous studies have failed to demonstrate an orexigenic effect of glucokinase (19). However, they did not specifically target the arcuate nucleus. In addition, they used methods that do not alter glucokinase activity specifically and that are known to alter food intake via other mechanisms (33). These effects on food intake are unlikely to be secondary to changes in circulating glucose or insulin levels, since neither fasting nor fed glucose and insulin levels were changed in the iARC-GK animals compared with those in the iARC-GFP animals.

Since glucokinase is an integral part of the glucose-sensing mechanism in the pancreas and specialized neurons (14, 17), we hypothesized that arcuate nucleus glucokinase activity may specifically regulate the consumption of glucose rather than just energy. This was tested by allowing the rats access to a source of pure glucose, either alone or in the presence of chow. Following injection of CpdA, intake of glucose was increased in iARC-GK rats compared with controls, while intake of fructose was unaffected. This suggests that the increase in food intake depends on the presence of glucose in the food. When glucose and chow were presented together, glucose intake was enhanced, while chow intake was unaffected by injection of CpdA. We found a similar effect in long-term studies comparing iARC-GK animals with iARC-GFP animals. In this situation, in contrast to our previous results when only chow was available, there was no difference in either chow intake or body weight between the 2 groups, while glucose intake was increased in the iARC-GK group compared with that in the controls. This supports the hypothesis that the increase in food intake in animals with increased glucokinase activity is in part driven by an increased physiological drive for glucose consumption. This hypothesis is further supported by the finding that, in iARC-GKAS animals with reduced glucokinase activity in the arcuate nucleus, glucose intake was reduced but chow intake was unaffected when both were freely available.

Intracerebroventricular injection of glucose has been established to decrease food intake as part of the system of metabolic regulation of food intake (14, 19, 34). Our data would seem contrary to this effect. However, there are several glucose-sensing regions in the brain, both within and outside the hypothalamus. It is likely that activation of these regions following injection of glucose into the brain is substantially different to that resulting from glucose ingestion.

The metabolic regulation of food intake by glucose is well established (14, 19, 34, 35), while homeostatic taste-independent mechanisms promoting glucose intake have been postulated but not demonstrated in mammals. Such a mechanism would increase intake of foods rich in glucose. Throughout evolutionary history, such foods have been difficult to obtain, and glucose is a vital nutrient for some tissues, including the brain. Therefore, a mechanism to maximize intake of glucose-rich foods when available would be advantageous. A similar mechanism has been found recently in invertebrates (36). In rodents and humans, glucose intake leads to a coordinated response between homeostatic and hedonic pathways regulating sensing, reward, and feeding behavior (7, 37). This study suggests that, within the arcuate nucleus, glucokinase is an integral part of the homeostatic CNS glucose-sensing mechanism that regulates glucose appetite. Our data does not exclude the possibility that other nuclei that express glucokinase within the hypothalamus or brain stem have a role in regulating glucose intake or homeostasis, as the effect of altered glucokinase activity in these regions was not tested. Data from invertebrate, rodent, and human studies suggest that food intake is regulated around a fixed macronutrient ratio (38, 39). Our results suggest that arcuate nucleus glucokinase regulates the set point for glucose and that altering arcuate nucleus glucokinase activity alters the set point for glucose consumption (40). Such a set point would explain the normalization in body weight gain in the iARC-GK and iARC-GKAS animals given access to glucose. The iARC-GK animals were able to sate their glucose appetite without intake of other nutrients. The glucose solution has a lower energy content per unit of glucose and thus the overall energy intake of these rats was reduced compared with those that only had access to chow. This same effect was observed with the controls in the rAAV-GKAS study. This mechanism would be specific for glucose, since other monosaccharides, for example, fructose, are not substrates for glucokinase and thus would not activate this pathway. Therefore, the maintenance of a glucose source in the diet of obese individuals may be important to satiate glucose appetite, given that hypothalamic glucokinase expression is elevated in animal models of obesity (32). This homeostatic system may explain the effects of foods with different glycemic index values on weight loss (41). Foods that have a high glycemic index value, which release large amounts of glucose quickly, would activate the arcuate glucokinase pathway. This would encourage further food intake. Foods with a low glycemic index value would not
activate this system, and so further intake would not be stimulated. It is possible that the recent rise in fructose consumption in Western-type diets may not satiate the glucose appetite, resulting in consumption of more calories (42).

Possible downstream signaling mechanisms were examined. The expression and activity of AMPK in the hypothalamus was unaffected by increased glucokinase activity in the arcuate nucleus. This suggests that AMPK is not an important mediator of the observed changes in food and glucose intake and increasing arcuate nucleus glucokinase activity does not change cellular energy availability (25). This is not surprising, since, as in β cells, energy production via glycolysis is dependent upon hexokinase I and not glucokinase (hexokinase IV). A slight increase in hypothalamic fatty acid synthase expression occurred in iARC-GKs rats. This may be a part of the mechanism underlying the increased food and glucose intake observed in the iARC-GK rats (27). The increase in FAS expression could be the result of increased food and glucose intake (25). This is not surprising, since, as in β cells, energy production via glycolysis is dependent upon hexokinase I and not glucokinase (hexokinase IV). A slight increase in hypothalamic fatty acid synthase expression occurred in iARC-GKs rats. This may be a part of the mechanism underlying the increased food and glucose intake observed in the iARC-GK rats (27).

The increase in FAS expression could be the result of increased ChREBP activation (43), driven by increased glucose-6-phosphate concentrations (44). Although activation of ChREBP would also be expected to increase ACC expression (43), this was not observed.

The subcellular localization and close proximity of glucokinase and membrane-bound K<sub>ATP</sub> channels may allow glucokinase and K<sub>ATP</sub> channels to function as a glucose-sensing complex (14). This would allow glucokinase to alter local ATP levels and change K<sub>ATP</sub> channel activity, without affecting cellular ATP. Such compartmentalization of glycolytic enzymes, glycolysis, and ATP production has been demonstrated in a variety of cells, including neuronal cells (45, 46). Injection of the K<sub>ATP</sub> channel inhibitor glibenclamide resulted in the same response as increased glucokinase activity in the arcuate nucleus. Injection of diazoxide, a K<sub>ATP</sub> channel activator, produced the opposite effect. In addition coadministration of diazoxide blocked the increase in glucose intake stimulated by CpdA. These results are supportive of a role for K<sub>ATP</sub> channels in the actions of glucokinase in the arcuate nucleus. K<sub>ATP</sub> channels are expressed in a small number of neurons, which do not express glucokinase (16). It is possible that the effects of glibenclamide and diazoxide on food and glucose intake are being mediated by these neurons rather than glucokinase-expressing neurons. However, given the effects that closely parallel those of increased glucokinase activity in the arcuate nucleus and the well-characterized coupling between glucokinase and K<sub>ATP</sub> channels (16, 17, 32, 47, 48), K<sub>ATP</sub> channels appear a likely mechanism for transducing the effects of glucokinase on glucose appetite via a mechanism analogous to glucose-stimulated insulin release in the pancreas (14). These results are in keeping with activation of hypothalamic K<sub>ATP</sub> channels by leptin (49, 50) and the observation that mice with targeted deletion of Kir6.2 have increased food intake (51). These findings also have important implications for the potential use of glucokinase activators and sulfonylureas in the treatment of type 2 diabetes. The increased food intake in response to arcuate nucleus glibenclamide administration could explain the increase in body weight seen in patients treated with sulfonylurea class agents (52).

The mechanism linking glucokinase to increased glucose intake is also likely to require activity of P/Q calcium channels. Injection of the specific P/Q channel blocker ω-agatoxin IVA completely blocked the increase in glucose intake stimulated by CpdA. Injection of the L-channel blocker nifedipine was without effect on CpdA-stimulated glucose intake. P/Q channels are expressed presynaptically throughout the CNS and are important in neurotransmitter release (53). Activity of these channels has been shown previously to mediate the release of NPY from hypothalamic slices (54).

A possible cellular mechanism underlying the changes in food and glucose intake is increased activity of arcuate nucleus NPY neurons, since there were changes in release of NPY in response to glucose from ex vivo hypothalamic slices from iARC-GK rats. As has been reported previously (55), increasing glucose concentrations in the artificial CSF (aCSF) resulted in reduced NPY release from control hypothalami. In contrast, there was no decrease in NPY release from iARC-GK hypothalami with increasing glucose concentrations. These results were mimicked by the treatment of ex vivo hypothalamic slices with either CpdA, a glucokinase activator, or glibenclamide, which blocks K<sub>ATP</sub> channels. Both of these treatments resulted in increased NPY release from hypothalamic explants. Conversely, treatment of hypothalamic slices with diazoxide, which opens K<sub>ATP</sub> channels, reduced NPY release and blocked the release of NPY stimulated by CpdA. A role for NPY in mediating this effect is supported by the finding that the increase in glucose intake stimulated by injection of CpdA into the arcuate nucleus could be attenuated by the administration of either a specific NPY Y1 receptor or NPY Y5 receptor antagonist. This suggests that NPY acting on both the Y1 and Y5 receptors is involved in the regulation of glucose intake (16, 48). NPY has been shown to preferentially increase carbohydrate intake compared with other dietary constituents (56). These findings also fit with the increased NPY expression, but unaltered POMC expression, in mice with targeted deletion of Kir6.2, a component of K<sub>ATP</sub> channels, suggesting that the K<sub>ATP</sub> channel may play a role in the regulation of NPY signaling in the arcuate nucleus (51). The finding of Parton et al. that transgenic mice expressing K<sub>ATP</sub> channels unresponsive to ATP in POMC neurons had normal body weight (57) further supports this potential mechanism.

It is therefore plausible that food with high levels of glucose causes increased activation of arcuate nucleus NPY neurons, via glucokinase, K<sub>ATP</sub> channels, and P/Q voltage-gated calcium channels, promoting further intake of that food in a positive feedback loop (Figure 6). This system would promote the intake of glucose-rich foods above that of those containing lower levels of glucose.

Overall, these results suggest that a requirement for glucose, rather than calories alone, partly drives food intake and that arcuate nucleus glucokinase can regulate this specific intake of glucose. In the absence of a source of pure glucose, the increased physiological drive for glucose, resulting from greater glucokinase activity in the arcuate nucleus, drives the intake of mixed nutrient foods. This hypothalamic system may form part of the postigestive reward pathway of glucose (7, 58) or form a separate homeostatic regulatory system regulating glucose appetite, analogous to that described recently in invertebrates (36). A role in taste-driven intake is less likely, as this is thought to be more responsive to fructose and sweeteners (59), which do not appear to activate this glucokinase-based glucose-sensing system, maximizing intake of what was until recently a rare resource. Evidence supports the existence of this pathway in humans. Patients with mutations that decrease glucokinase activ-
ity have reduced body weight (60). Conversely, patients who have mutations associated with increased activity of the neuroendocrine form of glucokinase have increased body weight (61).

Our results suggest that arcuate nucleus glucokinase is acting as part of a glucose-sensing system, analogous to that in the β cell, as part of a central macronutrient regulatory system. This glucose-sensing system regulates glucose appetite and energy homeostasis. Glucose from food enters arcuate nucleus neurons by GLUT-2 and is phosphorylated by glucokinase to glucose-6-phosphate, which is linked to closure of K<sub>ATP</sub> channels; this induces neuronal depolarization and entry of calcium via P/Q calcium channels, leading to release of NPY. Increased arcuate nucleus glucokinase activity could lead to a positive energy balance and increased preference for glucose. Diet-induced obese and diet-induced obese-prone rats have increased arcuate nucleus glucokinase mRNA expression, suggesting that this system may play a role in the development of obesity (32). This mechanism may explain the observation that diets high in carbohydrate are associated with weight gain in mice (62) and why low glycemic index diets produce weight loss (41). It also provides a possible CNS mechanism to explain the often-described phenomena of the “sweet tooth” and carbohydrate craving, particularly for high glycemic index foods.

### Methods

**Animal housing conditions.** Adult male Wistar rats (230–280 g, Charles River) were housed in single cages and maintained under a controlled environment (temperature 21°C–23°C, 12-hour light/12-hour dark cycle, lights on at 07:00 hours), with ad libitum access to chow (RM1; SDS Diets) and water, except where stated.

**Glucokinase plasmid construction.** Full-length glucokinase cDNA was isolated and amplified by PCR from pCMV4.GKB1 encoding full-length glucokinase cDNA (gift from Mark Magnuson, Vanderbilt University Medical Center, Nashville, Tennessee, USA) and cloned into pTR-CGW (gift from Joost Verhaagen, Nederlands Herseninstituut, Amsterdam, The Netherlands) (63).

**rAAV production.** rAAV was produced, isolated, and purified as described previously (63). The titer for rAAV-EGFP was 5.04 × 10<sup>12</sup> genome particles per ml, that for rAAV-GK was 2.96 × 10<sup>12</sup> genome particles per ml.

**rAAV microinjections.** Animal surgical procedures and handling were carried out as previously described (63). 1 μl of either rAAV-GK or rAAV-GFP was stereotaxic injected according to coordinates of Paxinos and Watson (64) (3.4 mm caudal, ± 0.5 mm lateral to the bregma, and 9.5 mm below the surface of the skull).

Starting body weights for studies with normal chow were as follows: iARC-GFP, 270.08 ± 3.04 g, and iARC-GK, 273.60 ± 2.75 g. Starting body weights for long-term studies with normal chow and 10% w/v glucose were as follows: iARC-GFP, 428.63 ± 11.58 g, and iARC-GK, 449.25 ± 5.60 g. Acute 24-hour normal chow and sugar intake feeding studies commenced at lights on (19:00 hours). Rats were killed during the early light phase by decapitation. Hypothalami, whole brains, and weighed interscapular BAT were collected and stored at −70°C. Correct targeting of rAAV to the arcuate nucleus was confirmed experimentally in several ways. Brains from one group of animals (n = 15) injected with rAAV-GK were subjected to in situ hybridization to detect glucokinase and assessed for localization to the arcuate nucleus. In all animals, expression of glucokinase was found to be localized to the arcuate nucleus. Brains from one group of rats injected with rAAV-GFP were subject to either in situ hybridizations for expression of WPRE (n = 12), a component of the rAAV construct, or immunocytochemistry for GFP (n = 2). In all of these animals, expression was found to be limited to the arcuate nucleus. This process was repeated in a second group of animals injected with rAAV-GK or rAAV-GFP. The brains of all of these animals were subject to in situ hybridization for WPRE expression. In all of these animals, expression was found to be limited to the arcuate nucleus.

**Cannulation.** Intra-arcuate cannulation was performed as previously described (22). Cannulas (Plastics One) were stereotaxically implanted unilaterally into the arcuate nucleus. At the end of the injection studies, cannula placement was confirmed histologically in all animals. One microliter of Indian ink was injected via the cannula. The brains were removed and fixed in 4% formaldehyde, dehydrated in 40% sucrose, and frozen in isopentane cooled in liquid nitrogen. Brains were sliced into 15-μm coronal sections on a cryostat (Bright), and correct arcuate nucleus placement was determined by microscopy according to the position of the Indian ink. All cannula were correctly targeted to the arcuate nucleus.

**Injection of pharmacologic agents.** Rats received intra-arcuate injections at between 09:30 and 10:30 hours with either drug or vehicle control on 2 separate days, 72 hours apart. Rats were fasted overnight for studies when diazoxide was administered alone; all other studies were in ad libitum–fed animals. All compounds were administered in a 1 μl volume over a period of 1 minute. Doses were based on previous experiments (18, 65, 66). Data are presented as food intake and glucose intake relative to those in corresponding rats treated with control during the crossover study.

**Calcium channel studies.** Rats received intra-arcuate injections at between 09:30 and 10:30 hours, with study days being at least 72 hours apart. First, rats received an intra-arcuate injection of 5 nmol nifedipine, 1 μg α-agatoxin IVA, or saline in random order. Fifteen minutes later, they received an intra-arcuate injection of either 0.5 nmol CpdA or vehicle in random order, and glucose intake was subsequently measured. All compounds were administered in a 1-μl volume over a period of 1 minute. Doses were based on previous experiments (67–69).

**NPY antagonist studies.** Rats received injections at between 09:30 and 10:30 hours, with study days being at least 72 hours apart. First, rats received an intra-peritoneal injection of 10 mg/kg BMS-193885, 10 mg/kg CGP-71683, or vehicle in a volume of 100 μl in random order; the antagonists were chosen as they are able to cross the blood-brain barrier. Fifteen minutes later, they received an intra-arcuate injection of 5 nmol CpdA or vehicle in random order, and glucose intake was subsequently measured. Intra-arcuate injections were administered in a 1-μl volume over a period of 1 minute. Doses were based on previous experiments (70, 71).

**Glucokinase activity assays.** Sections 880-μm thick were cut from frozen brains using a freezing Shandon sled microtome and mounted on slides. Slides were kept on dry ice. Arcuate nucleus, VMN, and PVN were removed using a 22-gauge neuro punch (Fine Science Tools) (64). Nuclei were homogenized in 200 μl ice-cold buffer containing 0.0107 M MgCl<sub>2</sub>, 5 mM sodium EDTA, 0.15 M KCl, and 0.07% w/v 2-mercaptoethanol. The homogenate was centrifuged at 16,000 g for 40 minutes.
at 4°C. Glucokinase activity was determined spectrophotometrically using an NADP+-coupled assay with glucose-6-phosphate dehydrogenase. Twenty microliters of homogenate was added to 1 ml reaction reagent containing 100 mM GlyGly, 45 μM 5-thio-d-glucose-6-phosphate, 1 M MgCl₂, 0.5 mM 3-O-methyl-N-acetylglucosamine, 200 mM ATP, 12.5 mM NADP, 2 M glucose, and 0.4 units glucose-6-phosphate dehydrogenase (type IX, from Baker’s yeast). Each reaction was undertaken in triplicate. The reaction was incubated at 37°C for 1 hour. Absorbance at 340 nm was measured and compared with a standard curve for glucokinase. Sample protein concentration was determined by BCA protein assay (Pierce).

AMPK activity assay. AMPK assays on hypothalami were undertaken as described previously (25).

In situ hybridization. In situ hybridization using antisense glucokinase cDNA riboprobe corresponding to nucleotides 15–116 of rat glucokinase was performed on 15-μm frozen brain sections.

Hormone and glucose assays. Plasma insulin and leptin concentrations were determined by enzyme-linked immunosorbent assay (Crystal Chem), and plasma glucose was determined by a glucose oxidase assay kit (Randox) using the manufacturer’s protocol.

Body composition. Body composition was calculated as previously described (72).

Quantitative PCR. Quantitative PCR was performed on hypothalamic cDNA using TaqMan Gene Expression Assays (primer assay ID: glucokinase Rn00561265_m1, Ampk Rn00576935, Acc Rn01456589_m1, Fas Rn01463550_m1, 18S 4310893E). mRNA expression relative to housekeeping gene (18S) was calculated according to the ΔΔCT method.

Northern blot. Expression of Ucp1 mRNA in interscapular BAT was measured by Northern blot with normalization to oligo dT.

Hypothalamic explant static incubation system. The static incubation system used was a modification of the method previously described to measure neurotransmitter release from hypothalamic explants in response to glucose or pharmacologic agents (57, 73). Following a 2-hour equilibration period in aCSF, hypothalamic explants were incubated for 45-minute periods in 600 μl aCSF containing 3, 8 (baseline), and 15 mM glucose in randomized order for experiments assessing glucose-stimulated neurotransmitter release in hypothalami from IARC-GFP rats and IARC-GKS rats. For experiments assessing hypothalamic neurotransmitter release following treatment with drugs, hypothalamic explants were incubated for 45 minutes in 600 μl aCSF (baseline control), followed by 45 minutes in 600 μl aCSF containing 30 μM CpdA (Mercck), 100 μM Glibenclamide (Sigma-Aldrich), or 200 μM Diazoxide (Sigma-Aldrich). Viability of the tissue was verified by 45-minute incubation in aCSF containing 56 mM KCl. Only hypothalami that showed secretion over baseline in response to 56 mM KCl were used in the analysis. At the end of each incubation period, supernatants were removed and tested for NPY or a-MSH release by radioimmunoassay, as described previously (74, 75).

Statistics. Results are shown as mean and SEM. A generalized estimating equation was used to compare cumulative data between control and treatment groups. Data from glucokinase activity assays and calcium channel blocker and NPY antagonist studies were analyzed using 1-way analysis of variance, followed by post-hoc Holm-Sidak test. A paired Student’s t test was used to compare food intake and glucose intake data from crossover studies. An unpaired 2-tailed Student’s t test was used to compare all other data. Significance was set at P < 0.05 for all analyses.

Study approval. All animal experiments were approved by the home office under the United Kingdom Animals (Scientific Procedures) Act 1986 (70/7229).

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