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Glucokinase as a Glucose Sensor in Hypothalamus - Regulation by Orexigenic and Anorexigenic Peptides

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1. Introduction

Glucose homeostasis requires hormonal and neural mechanisms in an attempt to get a normal functioning of the brain and of peripheral tissues. Blood glucose levels must be maintained within a physiological range depending of feeding and hormonal status, having the alterations of normoglycemic levels deleterious consequences. Hypothalamus plays a major role on feeding behaviour and energy homeostasis. It contains the called "satiety centre" and "hunger centre" located in ventromedial (VMH) and lateral hypothalamus (LH) respectively. These brain areas, besides others, may be altered by metabolic signals, such as changes in the electrical activity of neurons by direct application of glucose or by modifications of blood glucose levels. In this regard, glucose activates or inhibits neuronal activity, and both responses suggest the presence of glucose sensors in these brain areas.

Glucose sensors are molecular designs responsible for detecting and measuring glucose concentrations in the extracellular space. Thus, glucose sensor are presents in gut, endocrine pancreatic cells, portal vein, central nervous system and rare neuroendocrine cells, and they are responsible to avoid marked blood glucose oscillations, which permit to maintain glucose homeostasis.

First evidence of the existence of a glucose sensor system was reported in pancreatic beta-cells (Matschinsky 1990), constituted by glucokinase (GK). GK catalyses glucose phosphorylation with low afffinity and it is not inhibited by its product (glucose-6-phosphate), which allows increased glucose utilization as its concentration rises. Due to GK properties, the glucose catabolism rate is proportional to glucose levels in the extracellular space and for that reason GK is the major contributor to glucose sense, since catalyses the rate-limiting step of glucose catabolism. Interestingly, glucose transporter isoform 2 (GLUT-2) also with a high Km for glucose transport, has a different role since glucose transport occurs in both directions of the beta-cell membrane and glucose transport is 100-fold higher that the rates of glucose metabolism.

Our previous findings (Alvarez et al., 1996; Navarro et al., 1996) indicating the presence of GK together with GLUT-2 and glucagon-like peptide-1 receptor (GLP-1R) in the same cells

of hypothalamic areas implied in the control of feeding behaviour, suggest that a glucose sensor system may be present in those structures as discussed in the section 3.

GLP-1, together with others peptides, such as leptin, insulin, GLP-2, are anorexigenic peptides that acts in hypothalamic areas contributing to generate a state of satiety. By the contrary peptides orexigenic such as neuropeptide Y (NPY), orexin, galanin, ghrelin, etc, contribute to increase food intake. These effects may be the results of an accurate molecular crosstalk between the cells which secrete these peptides and the glucose sensing cells.

The importance of GK in the hypothalamic glucose sensing and its relation with anorexigenic and orexigenic peptides are discussed in this chapter.

2. Brain glucose sensor

Glucose is needed as an energy substrate but also as a signaling molecule in several processes. Glucoregulatory mechanisms are of primary functional concern to provide a continued glucose supply to the central neurons system and to face metabolic needs of peripheral tissues. Alterations of normoglycemic levels have deleterious consequences that increase the morbidity and mortality rates of the population.

In the 1950′s, the glucostatic hypothesis (Mayer 1953) postulated that glucose receptors exist in the hypothalamus and possibly in other central and peripheral regions involved in the regulation of food intake. Thus, glucose balance should be tightly regulated and taking into account the pancreatic glucose sensor concept, it would be reasonable to suggest the existence of a similar system in the brain that might modulate feeding behaviour and the release of counteregulatory hormones that defend against hypoglycemia. We proposed (Alvarez et al., 1996; Navarro et al., 1996) that the hypothalamus senses plasma glucose levels in a similar fashion to beta-cells, causing changes in the expression and secretion of neuropeptides (Yang et al., 2004). Our findings indicated that GK mRNA and protein are coexpressed together with GLUT-2 in the hypothalamus of human and experimental animals, mainly in areas involved in the control of food intake such as VMH and LH (Navarro et al., 1996; Roncero et al., 2000). Taking into account these studies, it have been addressed central role of GK as glucose sensor, reinforced by GLUT-2, the K^+_{ATP} channel subunits SUR1 and SUR2, as well as Kir6.2, in central glucose sensing (Jordan et al., 2010).

By analogy with the beta-cell, the general idea is that the glucose metabolism and variations in ratio ATP/ADP are the keys for neural sensing of glucose. However the ATP metabolism is a general mechanism for any electrical response, while neurons produce specific electrical responses. Others authors have proposed relationship between neuronal glucose-sensing and electrical responses (Figure 1). These hypothesis include electrogenic glucose entry, the existence of specific non-transporting detectors of extracellular glucose or glucose receptors and the possibility that glial cells would be responsible of glucose changes detection.

An example of electrogenic glucose entry to neuron is the mediated by the sodium–glucose co-transporters (SGLTs), where glucose is directly coupled to Na^+ ion movement. That entry is directly conditioned by extracellular glucose concentration. This mechanism is used in the secretion of GLP-1 by intestinal cells in response to glucose (Gribble et al., 2003) and in the excitability of glucose-response neurons of VMH and arcuate nucleus (ARC) (Yang et al., 1999) that will comment below.

Diez-Sampedro suggest the existence of a "glucose receptor" that mediates the changes in electrical activitiy (Diez-Sampedro et al., 2003). Thus, in some peripheral neurons, have been identified a glucose transporter protein (SGLT3/SLC5A4) that not transport glucose into the

cell, instead, this protein acted as a sensing receptor, converting elevations of extracellular glucose into Na^+-dependent depolarization of the membrane (Figure 1).

An additional hypothesis (Pellerin & Magistretti 2004) is that astrocytes could be the primary physiological detectors of extracellular glucose changes. This model proposed that rise in extracellular glucose induce glial lactate production which is transported from glia and into the neurons by lactate transporter (MCT1) and trigger the depolarization and excitation of neurons by closing the K^+_{ATP} channels (Burdakov & Ashcroft 2002) (Figure 1).

2.1 Glucose-inhibited (GI) and glucose-excited (GE) neurons

Claude Bernard showed the earliest evidence that the brain is involved in glycemic control since the lesion of the hypothalamus in dog, induced hyperglycemia (Bernard 1849). Later, Jean Mayer (Mayer 1953) proposed that hypothalamic cells could monitor plasma glucose variations and postulated that these cells transduced variations of glucose concentrations into electrical or chemical signals that control feeding behavior. Electrophysiological studies carried out in hypothalamic slices, demonstrated the existence of neurons able to modulate their firing activity in response to changes in extracellular glucose levels (Anand et al., 1964). These are glucose-excited (GE; previously called glucose-responsive) neurons, which increase their firing rate with elevation of glucose concentrations in extracellular, or glucose-inhibited (GI; previously called glucose-sensitive) neurons, which are activated by a decrease in extracellular glucose concentration or by cellular glucoprivation (Routh 2002; Yang et al., 2004).
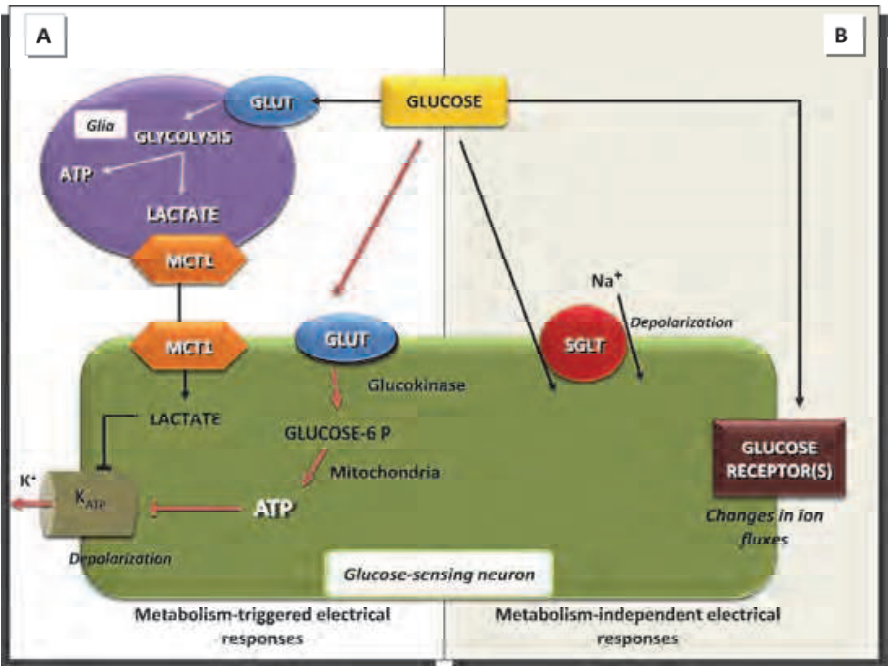

Fig. 1. Signalling pathways proposed to be involved in glucose sensing by hypothalamic neurons. A) Signalling pathways of glucose-metabolism-triggered electrical responses for neural sensing of glucose. Red lines indicate the canonical pathways share with beta-cell. Other signalling pathways could be mediate by previous glucose metabolization to lactate by glial cells. B) Pathways that triggers glucose metabolism-independent electrical responses. These pathways involved electrogenic glucose entry through sodium-glucose cotransporters (SGLT) or the presence of glucose receptors

Although both types of neurons are widely distributed in the brain, they are highly present in regions involved in the control of energy homeostasis and food intake such as hypothalamic nuclei and the brain stem (Adachi et al., 1995). Additionally it has been recently reported a novel glucose-sensing region in the medial amygdalar nucleus (Zhou et al., 2010).

The existence of glucose-regulated neurons has been studied by intravenous or intracerebroventricular injections of 2-deoxyglucose or 5-thio-glucose and posterior electrophysiological recordings or by the detection c-fos-like immunoreactivity (which is highly expressed in activated neurons). Using these approaches, responsive neurons have been found in the VMH, paraventricular (PVN) and LH, ARC, parabrachial nucleus (PBN), nucleus tractus solitarius (NTS), area postrema (AP), dorsal motor nucleus of the vagus (DMNX), and the region of the basolateral medulla (BLM) (Ritter & Dinh 1994; Dallaporta et al., 1999).

2.2 Electrophysiological pattern

Diverse signalling pathways are involved in the modulation of the electrical activity of hypothalamic neurons by glucose. On the one hand, glucose could alter neuronal electrical activity acting as energy substrate by influencing energy metabolism inside neuron and glia cells (Figure 1). On the other hand glucose could be considerer as an extracellular signalling messenger that could trigger specific glucose receptors controlling the membrane ion fluxes. Other possibility could be that glucose itself can be transported by electrogenic transporters (Figure 1). Which of these general mechanisms is dominant in different glucose-sensing neurons remains to be determined.

The mechanisms regulating GE cells have been more thoroughly understood and were assumed to employ a beta-cell glucosensing strategy. Thus, glucose enters the beta-cell via GLUT-2, facilitating its diffusion down a concentration gradient. Then GK phosphorylates glucose allowing for its entry into the glycolytic pathway that leads to an increase in the ATP. The consequent increase in the cytosolic ATP/ADP concentration ratio then closes the K^+_{ATP} channels. In the beta-cell, these channels are made up of Kir6.2/SUR1 subunits (Ashcroft & Rorsman 2004). Since open K^+_{ATP} channels generate hyperpolarization of the beta-cell and so dampen its excitability, their closure results in decreasing potassium outflow and depolarization with increased electrical activity. Actually, GK are the key glucose-sensing features of this scheme because they ensure that changes in extracellular glucose levels within the physiological range are converted to proportional changes in electrical excitability. This is due to the low affinity for glucose of GK, unlike the more ubiquitously expressed hexokinase I, and that GK is not inhibited by its product, glucose-6-phosphate.

The functional importance of GK in GE neurons was studied by pharmacological inhibition which decreased neuronal activity. *In vitro* studies on primary VMH cultures have shown that selective downregulation of GK leads to selective loss of glucose sensing and a decrease in cellular ATP concentration corresponding with an increase in K^+_{ATP} channel activation (Dunn-Meynell et al., 2002; Kang et al., 2006). This supports the model that inhibition of GK, in at least a proportion of GE neurons, reduces ATP production causing K^+_{ATP} channel opening and cell hyperpolarization. Recent findings indicate that although elements of canonical model used in beta-cell, are functional in some hypothalamic cells, this pathway is not universally essential for excitation of glucose-sensing neurons by glucose. In rats and

humans, the expression of GLUT-2, GK (Navarro et al., 1996; Roncero et al., 2000) and Kir6.2 was found in some but not all GE neurons (Kang et al., 2004) and were also present in non-glucose-sensing neurons (Lynch et al., 2000). For example, glucose-induced excitation of ARC neurons was recently reported in mice lacking Kir6.2, and no significant increases in cytosolic ATP levels could be detected in hypothalamic neurons after changes in extracellular glucose. Thus, the molecular support for the idea that all GE neurons rely on the beta-cell tools to sense glucose is currently inconclusive and possible alternative glucose-sensing strategies could include electrogenic glucose entry, glucose-induced release of glial lactate, and extracellular glucose receptors (Pellerin & Magistretti 2004; Burdakov et al., 2005).

Much less is known about GI neurons and whether these neurons follow the same glucose sensing strategy remains unclear (Burdakov & Lesage 2010). It has been proposed to involve reduction in the depolarizing activity of the Na^+/K^+ pump, or activation of a hyperpolarizing Cl^- current. Although it has been suggested that the metabolic model of glucose sensing applies to GI neurons, there is convincing evidence for metabolism-independent neuronal glucose sensing in GI neurons. As it has been mentioned, Kang group's (Kang et al., 2004) showed that some but not all glucose sensing neurons of the VMN express GK and SUR1, suggesting that other regulatory mechanisms must control glucose sensing in some of these neurons. Furthermore, Gonzalez et al (Gonzalez et al., 2009), used direct electrophysiological measurements of glucose sensing orexin neurons to show that metabolism independent glucose sensing exists in GI neurons. They found that GK inhibitors do not block glucose sensing in these neurons. However, despite the evidence for metabolism-independent as well as dependent glucose sensing mechanisms, the nature of the glucose sensing machinery in GI neurons remains unclear.

2.3 Hypothalamic distribution of GE and GI neurons

Hypothalamic glucose-sensing neurons comprise subgroups of cells in PVN, LH, ARC, VMH hypothalamic regions, and can be either electrically inhibited or excited by elevations in extracellular glucose (Anand et al., 1964; Oomura et al., 1969; Routh 2002; Wang et al., 2004). The distribution of GE glucose response neurons in the hypothalamus are confinated mostly to the VMH, ARC, and PVN (Dunn-Meynell et al., 1998; Silver & Erecinska 1998; Wang et al., 2004). However, GI neurons are found mainly in the LH (Oomura et al., 1974), median ARC, and PVN.

There are two major subpopulations of neurons in the ARC of the hypothalamus implicated in the control of feeding. One population contains pro-opiomelanocortin (POMC) and α-melanocyte-stimulating hormone (α-MSH) that are anorexigenic peptides and inhibits food intake. The second population of neurons contains agouti-related protein (AgRP) and neuropeptide Y (NPY) orexigenic peptides that stimulate food intake (Cone et al., 2001). GI neurons are present in the VMN in the ARC and in the LH and partly overlap with the orexigenic NPY/AgRP and possibly orexin neurons. GE neurons correspond in part to POMC neurons in the VMN and possibly in the ARC and POMC neurons in the LH (Mountjoy & Rutter 2007).

2.4 Effect of glucose concentrations on the brain glucose sensors

Some of the glucose-sensing neurons in the LH, ARC and VMH hypothalamic regions electrically respond to changes in glucose within the physiological ranges of glucose (Wang et al., 2004; Burdakov et al., 2005; Song & Routh 2005). As a general rule, the extracellular

concentration of glucose in the brain is 10–30% of that in the blood (Silver & Erecinska 1998). During euglycemia, brain glucose levels are 0.7-2.5 mM, and may be reached 5mM under severe plasma hyperglycemia. On the other hand, plasma hypoglycaemia can cause the brain glucose to fall to 0.2-0.5 mM. Changes in plasma glucose corresponding to meal-to-meal fluctuation (about 5–8mM) (Silver & Erecinska 1994) lead to changes in glucose concentration in the brain that it is expected to be in the range between 1 and 2.5mM (Routh 2002). It is also noteworthy that glucose-sensing neurons located near regions with a reduced blood–brain barrier for example, the median eminence region that neighbors the hypothalamic ARC (Elmquist et al., 1999), may be exposed to a much higher glucose concentrations than other brain cells, even approaching those present in the plasma. If this is the case, for glucose-sensing neurons of the ARC, high glucose concentrations (greater than 5 mM) may well comprise a physiologically relevant stimulus (Fioramonti et al., 2004) . In this way, it has been described in the ARC, the presence of GE and GI neurons responsive to glucose over either a low (0–5 mM) or a high glucose concentration range (5–20 mM); the latter are referred to as high GE (HGE) or high GI (HGI) neurons, respectively (Fioramonti et al., 2004; Penicaud et al., 2006).

More details about the effect of glucose oscillations in the glucose sensor GK will be discussed in the section 4 of this chapter.

3. Glucokinase as part of a hypothalamic glucose sensor system

GK is a member of the hexokinase family (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) that catalyses the phosphorylation of glucose to glucose 6-phosphate. Hexokinases I, II and III have a high affinity for glucose, with low Km (Michaelis constant) values in the micromolar range. GK or hexokinase type IV has a low affinity for glucose; it is not inhibited by physiological concentrations of glucose 6-phosphate, and has a molecular mass of about 50 kDa (Iynedjian 1993)

For years, the liver and the pancreatic islets were considered to be the only tissues in which GK activity could be detected. Later on, GK mRNAs of appropriate sizes were found in the corticotroph anterior pituitary cell line AtT-20, in rat pituitaries (Hughes et al., 1991) and in brain and intestine of the rat, but no enzyme activity was reported in neither (Jetton et al., 1994; Alvarez et al., 1996; Navarro et al., 1996). However, Roncero et al 2000 described that GK gene expression in rat brain gave rise to a protein of 52 kDa, with a high Km phosphorylating activity. Brain GK showed kinetic properties similar to those previously reported for the enzyme of hepatic or pancreatic islet origin. It has a high apparent Km for glucose (8.9–15 mM) and displays no product inhibition by glucose 6-phosphate. The contribution of GK to the total glucose phosphorylating activity was 40–19% in different cerebral regions, measured with a radiometric assay, and of 25–14% as determined by a spectrophotometric method.

The presence of tissue-specific promoters in the GK gen allows differential regulation. The upstream promoter, now rightly called neuro-endocrine promoter to distinguish it from the hepatic promoter (Iynedjian et al., 1996; Levin et al., 2004), is functional in beta-cells and in the brain (Magnuson & Shelton 1989; Roncero et al., 2000), while the downstream promoter is used only in liver . GK levels in beta-cells appear to be controlled by glucose, probably through a post-transcriptional mechanism (Iynedjian 1993; Matschinsky et al., 1993). In contrast, the liver-specific promoter is mainly affected by insulin and glucagon, which explains the extraordinary transcriptional regulation by the nutritional state (Iynedjian 1993).

The short-term regulation of GK activity involves several mechanisms: Long-chain fatty acyl-CoAs have been shown to be allosteric competitive inhibitors in vitro of the liver enzyme (Tippett & Neet 1982) and human beta-cell GK (Moukil et al., 2000). Glucose regulates the activity of GK through a "mnemonic" mechanism, which increases the activity of liver GK in the presence of high levels of glucose and decreases it when the glucose level is low (Cornish-Bowden & Storer 1986). GK activity in the liver is also regulated by the GK regulatory protein (GKRP), which behaves as a competitive inhibitor of GK. In the presence of fructose 6-phosphate GKRP binds to GK and inhibits its activity, whereas fructose 1-phosphate prevents the formation of the complex GKRP-GK (Van Schaftingen 1989). GKRP is not only a protein that binds and inactivates GK, but also regulates the translocation of GK between the cytoplasm and the nucleus. When glycogenolysis and/or gluconeogenesis are activated, the concentration of fructose 6-phosphate increases in the liver and produces the inhibition of GK, which facilitates the release of glucose by the liver. When carbohydrates are present in the diet, fructose is phosphorylated to fructose-1 phosphate, which favours glucose utilization by the liver (Agius & Peak 1993; Toyoda et al., 1995; Shiota et al., 1999). Thus, in the liver, the subcellular translocation of GK regulates the enzyme activity in accordance with the metabolic needs of the cells. It is therefore accepted that translocation of GK to the nucleus at low glucose concentrations needs GKRP as an anchoring protein that allows transport through the nuclear pore complex. However, it is unclear whether the nuclear export of GK requires GKRP. In pancreatic islets, GK activity may also be regulated by a protein, in a similar way to that described in hepatocytes (Malaisse et al., 1990).

Subsequent research has also identified several GK binding proteins that could act as cytoplasmic binding proteins. They include a dual-specificity protein phosphatase, the glucokinase-associated phosphatase (Munoz-Alonso et al., 2000), and the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (Baltrusch et al., 2001). Other studies indicate that a minor fraction of GK and GKRP may be integrated in a multienzyme complex, including the pro-apoptotic protein BAD, and become associates with mitochondria in hepatocytes (Arden et al., 2006). These data suggest the existence of different pools of cytoplasmic GK with specialized metabolic functions (Arden et al., 2006). It has also been reported that AMP-activated protein kinase (AMPK) inhibits GK translocation from the nucleus.

In pancreatic beta-cells GK is considered to be a true glucose sensor (Matschinsky 1990) involved in glucose-dependent insulin release. The GK glucose sensor concept is supported by the fact that GK mutations are responsible for some types of maturity-onset diabetes of the young-2 (MODY-2) (Vionnet et al., 1992) and for persistent hyperinsulinaemic hypoglycaemia in infancy (PHHI) (Christesen et al., 2002). In addition, as noted above, targeted disruption of the GK gene in the beta-cells of mice produces severe alterations in insulin release (Grupe et al., 1995).

3.1 Expression, activity and localization of hypothalamic GK and glucokinase regulatory protein (GKRP) in the hypothalamus

Several brain areas, such as the LH and VMH and the dorsomedial medulla oblongata, including the NTS and the motor nucleus of the vagus, modulate glucose homeostasis in the liver and pancreas (Oomura & Yoshimatsu 1984). As previously cited, glucose is mainly excitatory in the VMH and inhibitory in the LH and NTS (Oomura & Yoshimatsu 1984), suggesting the presence of glucose sensors in neurons of these brain areas. Indeed, stimulation of glucoreceptors present in the neurons of the VMH, promotes the release of

the counter-regulatory hormones, catecholamines and glucagon, which defend the organism against glucopenia (Borg et al., 1995).

Our findings, indicate that GLUT-2, GK and GLP-1R are expressed in the same cells of the rat and human hypothalamus (Alvarez et al., 1996) (Figure 2), and are located in areas involved in the regulation of energy homeostasis, feeding behaviour and glucose metabolism. It is noteworthy that in the brain, GLP-1 contributes to reducing food intake (Navarro et al., 1996; Turton et al., 1996), and the co-localization of those three components in hypothalamic neurons suggests that a glucose sensor system may be involved in the transduction of signals required to produce a state of satiety.

As we previously cited, GK activity may also be regulated by GKRP, acting in accordance with the metabolic needs of the cells (Van Schaftingen et al., 1984; Shiota et al., 1999; Roncero et al., 2004). Interestingly, we reported the coexpression of GK and GKRP in both rat and human brains (Roncero et al., 2000; Alvarez et al., 2002; Roncero et al., 2004), as well as GKRP interacting with GK in the presence of fructose-6-phosphate, which suggests that both are active and both may participate in the glucosensing process in the central nervous system (Alvarez et al., 2002).
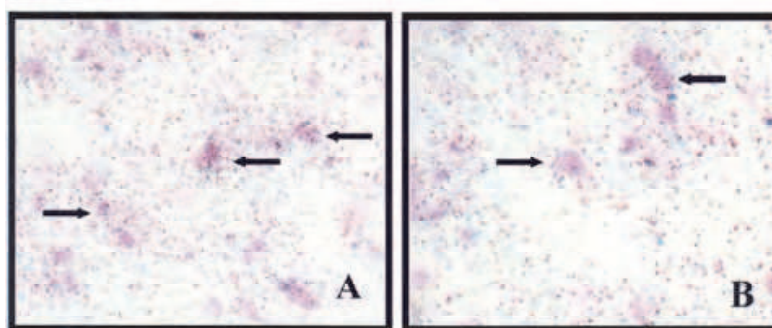

Fig. 2. In situ hybridization histochemistry of GLP-1R and GLUT-2 or GK mRNAs. **A** double-labeling of GLP-1R and GLUT-2 mRNAs in the VMH. **B** Double-labeling of GLP-1R and GK mRNAs in the VMH. Silver grains indicate the localization of either GLUT-2 or GK mRNAs. Blue reaction product indicates labelling of GLP-1R mRNAs. (Microphotographs suministrated by Dr. J. Chowen)

GK and GKRP have been found in foetal pancreas and liver (Vandercammen & Van Schaftingen 1993; Garcia-Flores et al., 2002). Also in foetal hypothalamus GK mRNA was present in day 18 of development (Sutherland et al., 2005). Our result concluded that GK and GKRP were functionally active before birth in the rat brain. Both proteins colocalised in the same cells of hypothalamus and the cerebral cortex of 21-day-old foetuses (F-21) (Roncero et al., 2009) (Figure 3).

The presence of GK mRNA was confirmed in approximately 50% of GE and GI neurons in the VMH. Some of these neurons also contained mRNAs for subunits of K^+_{ATP} channels (Kang et al., 2004). The use of specific small interfering RNA (siRNA) to knock-down GK in primary cultures of neurons from the rat VMH, abolished the responses to glucose (Kang et al., 2006). Together, these findings supported the idea that GK was the glucose sensor of at least a fraction of VMH neurons, functioning as the rate determining enzyme of glucose metabolism and controlling neuronal excitability. In the VMH, GK mRNA was shown to be expressed in neurons synthesizing POMC and NPY/AgRP, which play critical roles in neural pathways involved in the regulation of food intake and energy expenditure.
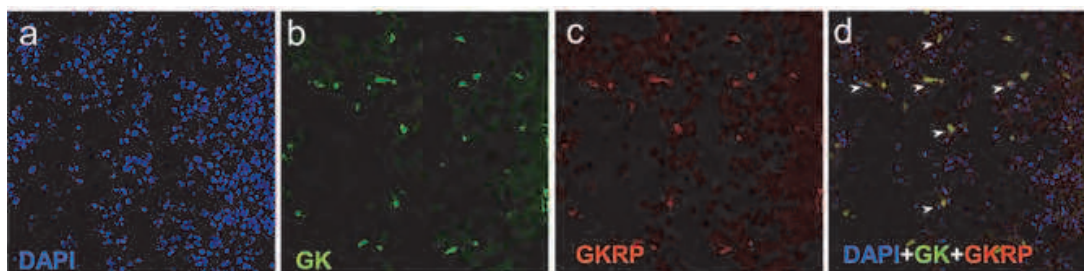
Fig. 3. GK and GKRP detection in the hypothalamus. Inmunofluorescent detection of GK (green b) or GKRP (red c) proteins in VMH from twenty-one-day old foetuses. Nuclear staining (a) was carried out in blue with 4,6-diamid-o-2-phenylindole (DAPI). The arrows (d) indicate the double positive cells for GK and GKRP.

4. Regulation of hypothalamic GK by glucose or regulatory peptides

The glucose-sensing neurons have receptors for orexigenic and anorexigenic neuropeptides that may modulate feeding behaviour through a glucose sensor system (Schuit et al., 2001).

The effects of orexigenic and anorexigenic peptides and glucose on GK gene expression were tested (Sanz et al., 2007) in GT1-7 immortalized hypothalamic neurons, which are glucose-sensing cells (Lee et al., 2005) able to respond to glucose deprivation or high-glucose levels, as well as having intrinsic GK activity. Neither the promoter activity of the GK gene in transfected GT1-7 cells nor the endogenous GK gene expression were modified by the action of different concentrations of glucose or GLP-1, leptin, or NPY in the extracellular space. However, GK enzyme activities were modified by these peptides.

Using hypothalamic slices in culture, instead cell line, which it is a better physiological model, the effect of glucose orexigenic and anorexigenic peptides on GK activities in VMH and LH areas were assayed. In the VMH there was a tendency for GK activities to increase as glucose rose in the extracellular media and high-GK activity was found at lower glucose concentrations in the LH. These findings could reflect a different type of behavior for the GE and GI neurons located in the VMH and the LH, when challenged by different concentrations of glucose. This would be in agreement with the tendency of GK activity to increase with rises in glucose in the range of 0.5–20 mM that was reported in the VMH (Sanz et al., 2007), while neurons in LH were not excited by the higher concentrations of glucose but by lower concentrations of this hexose, as happens with GK activities (Sanz et al., 2007). The observed changes in GK activity in response to glucose in certain hypothalamic areas suggest that such activity would not only be tissue-specific but also even cell-specific in defined brain areas. Interestingly, GK activities were thus significantly lower in LH than in VMH at high glucose concentrations, but this effect was reversed by the presence of insulin. This distinctive response observed in LH at high glucose may be related to different functional activities of these two areas, rather than to other kind of effects. The data obtained in these studies indicate a different response to glucose levels in the VMH and LH and also that some orexigenic and anorexigenic peptides might modulate GK activities in neurons of these areas. Additionally, it suggests that in most of the cases modifications of hypothalamic GK occur at the enzyme activity level rather than in transcriptional expression.

5. Role of hypothalamic GK on the control of feeding behaviour and body weight

Coexpression of GLUT-2, GK and GKRP in areas involved in feeding behaviour (Roncero et al., 2000; Roncero et al., 2004) might play a role in glucose sensing, in which GK and GKRP made possible a real sensor activity. Furthermore the effects of anorexigenic and orexigenic peptides through its receptors in this system should facilitate the transduction of signals required to produce a state of satiety. In fact, we have reported experimental evidences that GLP-1 is an anorexigenic peptide and controls the glucose metabolism in the human hypothalamus areas involved in the regulation of feeding behaviour (Alvarez et al., 2005) (Navarro et al., 1996).

5.1 Anorexigenic and orexigenic peptides

Early suggestions that hypothalamus plays a major role on feeding behaviour and energy homeostasis were obtained after brain lesion and stimulation studies. Remembering these observations, such as electrical stimulation of VMH suppress food intake and that bilateral lesions of these structures induce hyperphagia and obesity, VMH was named as the satiety centre, while alterations of LH induced the opposite set of responses and thus was called the hunger centre. Also, the dorsomedial medulla oblongata, including the NTS and the motor nucleus of the vagus, are implied in these processes. Now, we know the existence of specific subpopulations of neurons involved in energy homeostasis that are included in neuronal pathways with anorexigenic and orexigenic biomolecules (table 1), that generate integrated responses to afferent stimuli related to modifications in metabolites or in fuels storage.

The VMH is the responsible for integrating peripheral signals of nutrient status and adiposity. For example, the ARC contains neurons secreting the NPY and POMC. These neuropeptides have opposite effects on energy homeostasis, since NPY increases food intake and inhibit energy expenditure, while POMC exerts the contrary effects (Adage et al., 2001). Other studies suggest that some VMH neurons are GABAergic, although there is not a clear relationship to glucose-sensing capacity. More recent findings show that most VMH neurons express the protein steroidogenic factor-1 (SF-1) and that SF-1-positives neurons have key roles in glucose homeostasis (Tong et al., 2007; Tsuneki et al., 2010). The LH cells contain the peptides orexins/hypocretins, which are not expressed anywhere else in the brain (de Lecea et al., 1998; Sakurai et al., 1998). Lack of orexin/hypocretin produces hypophagia and late onset obesity among others effects (Hara et al., 2001).

A number of peptidic hormones, previously thought to be specific to the gastroenteropancreatic system and later found also in the mammalian brain, have been shown to modulate appetite, energy, and body weight. They play these physiological effects together with other biomolecules such as NPY, opioid peptides, galanin, vasopressin, and GHRH (Bray 1992). Thus, feeding behaviour is controlled by the antagonist effects of anorexigenic and orexigenic biomolecules. The complex mechanisms underlying the abundance of such variety of feeding behaviour-modulating substances can be understood on the basis of, the specific role for each molecule as a regulatory mechanism in energy balance status, the specifity of macronutrient intake (carbohydrate, fat or proteins) and meal size control, and the shifts in feeding behaviour related to hormonal status, gender, age, and circadian rhythms (Leibowitz 1992).

It is accepted that cells of several hypothalamic nuclei detect circulating satiety signals and transmit this information to other brain areas. Anorexigenic and orexigenic biomolecules are

located in VMH, LH, PVN and ARC interacting one to others in a way that they may induce a characteristic feeding behaviour. Thus, peptide Y (Y3-36) is released from gastrointestinal tract postprandially, and acts on NPY Y_2 receptor in the ARC to inhibit feeding with a "long-term" effect (Batterham et al., 2002). On the contrary, other satiety signals induced by gut-brain peptides such as GLP-1, GLP-2 and CCK produced (Navarro et al., 1996; Turton et al., 1996; Rodriquez de Fonseca et al., 2000; Tang-Christensen et al., 2000) a "short-term" effect, while insulin and leptin (Batterham et al., 2002) inhibit the appetite by increasing the formation of POMC and reducing NPY action. In addition, ghrelin a peptide released by the stomach is stimulated before the meals to facilitate NPY action (Coiro et al., 2006).

ANOREXIGENIC		OREXIGENIC
CART	Leptin	AGRP
CCK	α-MSH	Galanin
CRH	Neurotensin	Ghrelin
GLP-1	Oxytocin	MCH
GHRH	Pro-opiomelanocortin	Noradrenaline
GLP-2	Peptide Y (Y 3-36)	NPY
IL-1β	TRH	Opioid peptides
Insulin	Serotonin	Orexin A and B
	Urocortin	

Table 1. Anorexigenic and orexigenic biomolecules implied in the control of feeding behaviour.

Intracerebroventricular or subcutaneous administration of GLP-1 produced a marked reduction of food intake and water ingestion. Exendin-4, proved to be a potent agonist of GLP-1R also decreased both food and water intake in a dose-dependent manner, while exendin (9-39) considered as an antagonist of the GLP-1R, reversed the inhibitory effects of GLP-1 or exendin-4 (Navarro et al., 1996; Turton et al., 1996; Rodriguez de Fonseca et al., 2000). Additionally, icv administration of GLP-1 to mice and rats produced a marked decrease of food intake but not of water ingestion. Surprisingly, this effect was avoided by the administration of exendin (9-39) (Tang-Christensen et al., 2000) .

5.2 Hypothalamic slices as a physiological model for the study of hypothalamic regulation by glucose and peptides

Some hypothalamic neuronal cell models have been generated from embryonic and adult animals to carry out molecular genetic analysis of hypothalamic neuronal function. Some examples are the GT1-7 and N1E-115 cell lines or the recently hypothalamic neuronal cell models generated by Belsham´s group (Dalvi et al., 2011). However, the use of organotypic slice cultures is an approach with many advantages. A broad range of studies shows that organotypic cultures retain many in vivo characteristics as regards, neuronal morphology, cellular and anatomical relations and network connections (Sundstrom et al., 2005). The advantages of the slices culture are the isolated and well defined environment of the in vitro preparations and in this way several brain areas have been studied (hippocampus, cerebellum, cortex, striatum, brain stem structures, spinal cord, retina and hypothalamus).

For these reasons, these cultures are increasingly been used as models to investigate mechanisms and treatment strategies for neurodegenerative disorders or exposure to neurotoxic compounds (Kristensen et al., 2003), traumatic brain injury (TBI) (Morrison et al., 2006) and neurogenesis (Lossi et al., 2009). Hypothalamic slices have also been frequently used for electrophysiological studies developed in vitro to evaluate the firing rate of specific neurons located in VMH and LH (Yang et al., 1999)

Most hypothalamic organotypic slice cultures have been derived from neonatal animals. In fact, slices of developing brain tissue can be grown for several weeks as so called organotypic slice cultures (Noraberg et al., 2005), but also adult rats have been used. In this sense our group have used short-term hypothalamic slices cultures, obtained from adult rat, to test the effect of different peptides on the gene expression and enzymatic activity in the VMH and LH areas (Sanz et al., 2007; Sanz et al., 2008). Thus, the hypothalamic organotypic cultures are a useful model for the study of the physiological effect of some peptides on specific hypothalamic nuclei, which could be dissected after the treatment and analyzed by enzymatic, molecular, biochemical or histological procedures.

5.3 Interactions between anorexigenic peptides and cerebral glucose metabolism in humans

By previous studies we know that GLP-1 is an anorexigenic peptide. The proglucagon gene is expressed mainly in the brainstem and hypothalamus, through a mRNA transcript identical to that produced in pancreas and intestine (Drucker & Asa 1988), which permits the formation of glucagon-related peptides in the brain. Furthermore, the hypothalamus and brainstem are the areas with the highest concentration of GLP-1 and its receptors (Uttenthal et al., 1992), suggesting that this peptide has a local function, as well as a more remote signalling role. The expression of GLP-1R gene gives rise to a protein with effects on the selective release of neurotransmitters (Mora et al., 1992), appetite and fluid homeostasis, as well as serves as a signal to reduce food intake (Navarro et al., 1996; Turton et al., 1996; Rodriquez de Fonseca et al., 2000). Also, the coexpression of GLP-1R with those of GLUT-2 and GK in the same cells suggest that these proteins play a role in glucose sensing (Navarro et al., 1996). A further step was to evaluate the GLP-1 effect on cerebral-glucose metabolism in control subjects by positron emission tomography (PET), using 2-F-18 deoxy-D-glucose (FDG). PET is an imaging technology in which compounds labelled with positron-emitting radioisotopes serve as molecular probes to identify and determine biochemical processes in vivo.

We used (Alvarez et al., 2005) intravenously injected FDG to trace the transport and phosphorylation of glucose in brain. FDG-6-phosphate is the end product of the process of glucose metabolism and it is not a substrate for subsequent metabolic reactions then it is retained in the cell proportionately to the rate of glycolysis. The activity measured reflects FDG transport and phosphorylation by the cells and, in the case of the hypothalamus and brainstem which contains cells expressing GLUT-2 and GK, may provide some information about brain glucose sensing.

PET imaging was sensitive to GLP-1 administration (Figure 4). Thus, this peptide significantly reduced cerebral glucose metabolism in the hypothalamus and brainstem as compared with the data obtained in normal control subjects without GLP-1 administration. These changes can be explained in terms of this peptide can enter the brain by binding to blood barrier-free organs such as the subfornical organ and the area postrema (Orskov et al., 1996). Also, it could be transported into the brain through the choroid plexus, which has a high density of GLP-1 receptors.
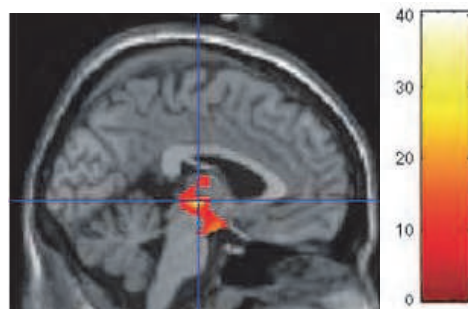
Fig. 4. PET of glucose metabolism in brains of human controls subjects, i.v. perfused with or without GLP-1 (0.75 pmol/Kg body weight for 30 minutes). The differences between both tests were of 6 months, and were projected on sagittal normalized brain MRI. PET imaging was sensitive to the peptide administration. Thus GLP-1 significantly reduced (p<0.001) cerebral glucose metabolism in the hypothalamus and brainstem as compared with the data obtained in normal control subjects without peptide administration.

Using PET technology we observed that iv administration of GLP-1 produced a significant reduction in carbohydrate metabolism in selective areas of the brain, including the hypothalamus and brainstem, both areas involved in feeding behaviour. Thus, the accumulation of FDG-6-phosphate into the cells serves to assess the facilitated transport and hexokinase phosphorylation of glucose, which, in the case of hypothalamus and brainstem cells containing GLUT-2 and GK, might facilitate the glucose-sensing process. These findings are of interest because glucoreceptive sites controlling food intake and blood glucose have been found in the medulla oblongata and mesencephalon of the rat (Ritter et al., 2000), in addition to those reported in the hypothalamus. Also, GLP-1 is expressed in human brain, and it most likely mediates the effect of GLP-1 on glucose metabolism in selective areas of hypothalamus and brainstem; it may also facilitate the process of glucose sensing in these areas. Because the reduced number of neurons involved in glucose sensing (Oomura et al., 1969; Ashford et al., 1990) , approximately 40% of cells in the VMH and 30% of cells in the LH, PET imaging offers a good procedure for identifying these cell signals in vivo more accurately compared with the more commonly used in vitro procedures.

These findings provide first evidences of the action of an anorexigenic peptide on glucose metabolism in the hypothalamus and brainstem, and might explain the satiety–induced effects of peripheral or central administration of GLP-1 and the central alterations produced by the iv administration of the peptide. Furthermore, open new doors for studying the effects of other regulatory peptides in subjects under control and pathophysiological situations.

6. Others hypothalamic metabolic sensors: AMPK structure and regulation

AMP-activated protein kinase (AMPK) functions as a cellular energy sensor being activated during energy depletion. The kinase is mainly activated by an increase AMP/ATP ratio. Activation of AMPK regulates a large number of downstream targets stimulating ATP-generating, catabolic pathways and inhibiting anabolic pathways (Hardie et al., 1998; Rutter et al., 2003).

AMPK is a heterotrimeric serine/threonine kinase consisting of a catalytic α-subunit encoded by 2 genes (α1 or α2), a β-subunit encoded by 2 genes (β1, β2) and a regulatory γ-subunit encoded by 3 genes (γ1, γ2, γ3). Different isoforms and alternative splicing of some

mRNAs encoding these subunits, give rise to large variety of heterotrimeric combinations (Hardie 2007; Viollet et al., 2009). It is known that some tissues can express several types of AMPK complexes.

AMPK can be regulated by an allosteric mechanism, through AMP binding to γ subunit, and by covalent phosphorylation at Thr^{172} located in the kinase domain of the α-subunit. The level of phosphorylation is also regulated by AMP through stimulation of upstream kinases and by inhibition of dephosphorylation by protein phosphatases. One of the identified upstream kinases of AMPK is the tumor-suppressor (Liver Kinase B1: LKB1). In neural and endothelial cells AMPK may be also activated by rises in cytosolic Ca^{2+} concentration. The calmodulin-dependent protein kinase kinase (CaMKK) can also activate AMPK by phosphorylation of Thr^{172}.

The AMPK is able to detect changes in cellular energy state that occur in response to variations in nutrient concentrations. Any cellular or metabolic stress that reduces ATP production or accelerates ATP consumption will increase the ratio of the ADP/ATP, which will be amplified by the action of adenylate kinase resulting in increased AMP/ATP with consequent activation of AMPK. Once activated, AMPK first directly affects the activity of key enzymes of glucose metabolism and fatty acids and at a second more long term regulates transcriptional control of the main elements involved in these metabolic pathways. The net result of the activation of AMPK will restore energy balance inhibiting anabolic pathways responsible for the synthesis of macromolecules. (Figure 5)
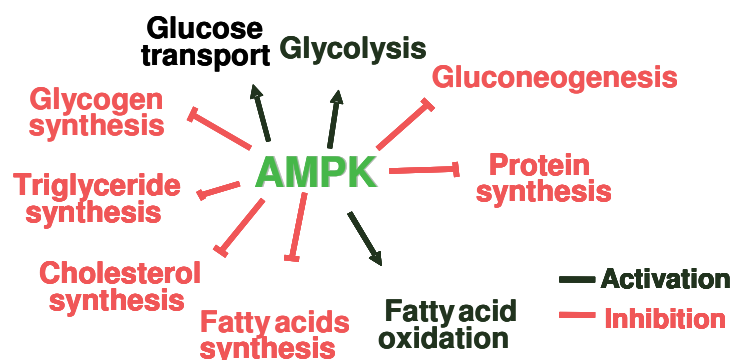

Fig. 5.AMPK regulation of downstream metabolic events. AMPK restores energy balance by activation of processes that produce energy and inhibition of those that consume energy.

6.1 Role of AMPK in hypothalamic glucose sensing

AMPK is broadly expressed throughout the brain with a mainly neuronal distribution (Turnley et al., 1999). Hypothalamic AMPK has been proposed to play a role in the central regulation of food intake and energy balance. In this way, fasting increases and re-feeding decreases AMPK activity in various hypothalamic nuclei (Minokoshi et al., 2004). Several studies have demonstrated that hypothalamic AMPK is regulated by blood glucose levels. Peripheral or central hyperglycaemia inhibits AMPK in several hypothalamic nuclei. Insulin-induced hypoglycaemia and inhibition of intracellular glucose utilization through the administration of 2-deoxyglucose both increased hypothalamic AMPK activity and food intake.

The use of catalytic subunit of AMPK knockout mice (AMPKα1$^{-/-}$ and AMPKα2$^{-/-}$) indicated that AMPKα1$^{-/-}$ mice has no metabolic alterations, whereas AMPKα2$^{-/-}$ mice showed insulin-resistance characterized by impaired insulin stimulated whole-body glucose

utilization and skeletal muscle glycogen synthesis and reduced insulin secretion. However, they showed no apparent changes in body weight and food intake (Viollet et al., 2009).

In the last years AMPK has been proposed as a cellular energy sensor that is able to assemble many regulatory signals and nutritional environmental changes, also involved in maintaining whole body energy balance (Hardie et al., 2006). Regulation of AMPK activity in hypothalamic areas involved in the control of feeding behaviours have been also described as a mechanism to detect nutritional variations including glucose levels (Mountjoy & Rutter 2007). Neurons implied in the control of food intake have as well glucose sensor that respond to fluctuations in extracellular glucose levels.

In general most cells will express glucose transporters that have high affinity for glucose; therefore ATP synthesis by glucose metabolism only stops when glucose levels are pathologically low. However, cells that have a glucose sensor mechanism, express GLUT-2 and GK. The presence of these proteins of high Km for transport and metabolism of glucose will allow that ATP synthesis decreases when decreases the concentration of glucose in the physiological range and this allows that in these cells the AMPK can be activated by low levels of glucose and inhibited by high glucose levels in the physiological range.

The hypothalamic AMPK role has been studied in vivo by expression of AMPK mutants: Inhibition of hypothalamic AMPK suppresses neuronal NPY/AgRP signalling and inhibit food intake. However, elevated AMPK increases NPY/AgRP expression, food intake and body weight (Minokoshi et al., 2004).

The role of AMPK in the two major subpopulations of neurons involved in the regulation of feeding was later analyzed by knock out of AMPKα2 in specific neurons of mice. The results showed that genetic deletion of AMPKα2 in hypothalamic POMC neurons (POMC AMPKα2-KO) developed obesity. However, AMPKα2 specifically knocked in AgRP neurones (AgRP AMPKα2-KO) exhibited an age-related lean phenotype (Claret et al., 2007). Those mice do not respond to changes in extracellular glucose. The unexpected results suggest that absence of AMPK in orexigenic AgRP neurons reduced body weight, whereas lack of AMPK in anorexigenic POMC neurons increased body weight. These results suggest a role for AMPK as a common glucose-sensor in these neurons (Claret et al., 2007). It is important to emphasize that GE neurons do not overlap completely with POMC neurons in the ARC while the GI neurons do not completely overlap with NPY/AgRP neurons. Those findings indicated the complexity of the functions of hypothalamic AMPK.

6.2 Regulation by orexigenic and anorexigenic peptides

Several reports indicate that AMPK is also regulated by several orexigenic and anorexigenic signals (Minokoshi et al., 2004). Thus, hypothalamic AMPK activity was inhibited in ARC and PVN by anorexigenic peptides as leptin and insulin by contrast, orexigenic peptides (ghrelin) increased specifically α2AMPK activity

In other hand, other studies reported AMPK-independent pathways. The effect of leptin were maintained in POMC α2AMPK-KO neurons and insulin depolarised AgRP neurons in AgRP α2AMPK-KO (Claret et al., 2007). The different results may be explained, in addition to the different techniques and experimental setups, by the presence of distinct subpopulations of GE and GI neurons with different neuropeptide phenotypes, responses to hormonal stimuli and, in some cases, different glucose-sensing mechanisms.

The downstream pathways of AMPK in the hypothalamus may involve the acetyl-CoA carboxylase (ACC)-malonyl-CoA-carnitine palmitoyltransferase 1 (CPT1) pathway and the
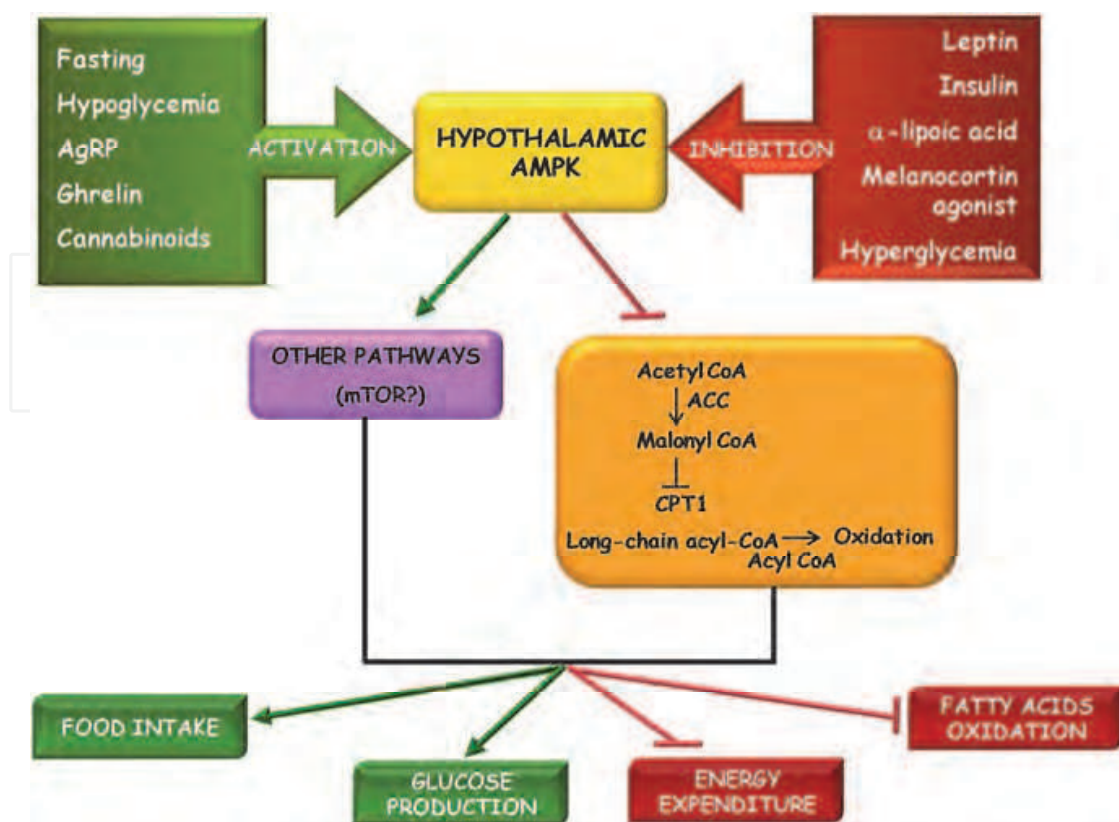
Fig. 6. Regulation of hypothalamic AMPK activity. Downstream targets involved in this signaling pathway. Several orexigenic and anorexigenic peptides regulate AMPK activity. Some of downstream targets of AMPK in the hypothalamus: ACC, acetyl-CoA carboxylase; CPT1, carnitine palmitoyltransferase 1. The effect of AMPK on the mammalian target of rapamycin (mTOR) pathway has not been directly proved in the hypothalamus.

mammalian target of rapamycin (mTOR) pathway. Activation of ACC, as a consequence of AMPK inhibition, would lead to increased intracellular malonyl-CoA levels, which would inhibit mitochondrial CPT1 and fatty acid oxidation.

The mTOR is another possible target of AMPK (Figure 6). AMPK inhibits mTOR signalling, thereby suppressing protein synthesis, which is an important pathway by which AMPK conserves cellular energy. The mTOR signalling pathway plays a crucial role in the regulation of food intake and body weight in the hypothalamus (Cota et al., 2006; Ropelle et al., 2008). The mTOR is colocalized with AgRP/NPY and POMC neurons in the ARC (Perrin et al., 2004). Fasting downregulates mTOR signalling, whereas re-feeding activates it.

Interestingly, hypothalamic AMPK also mediates the counter-regulatory response to hypoglycaemia, increasing the release of peripheral hormones such as corticosterone, catecholamines and glucagon (Han et al., 2005; McCrimmon et al., 2006). Thus AMPK is not only a peripheral or a central mediator, but also a key enzyme in coordinating the interaction between peripheral and central energy regulation.

It is generally accepted that in peripheral organs, fuel overabundance alters the activity of metabolic sensors (decreased AMPK and increased the mTOR and its down-stream target the S6Kinase (p70S6K)) causing insulin resistance (Kola et al., 2005).

AMPK and mTOR activity respond to changes in glucose and other nutrients in hypothalamic centres involved in control of feeding and deregulation of this signalling pathways might be

implied in the develop of obesity and diabetes type 2. GLP-1 treatment to type 2 diabetic subjects normalizes fasting levels of blood glucose and decreases glucose levels after ingestion of a meal (Niswender 2010). GLP-1 is able to induce several effects that contribute to feeding behaviour. Preliminary data from our laboratory also suggest that, at least some of those effects, might mediate through regulation of AMPK and p70S6K in VMH and LH.

7. Conclusions

Glucoregulatory mechanisms are of primary functional concern to provide a continued glucose supply to the central nervous system and to face metabolic needs of peripheral tissues. Glucose sensors are molecular designs that accurately measure glucose concentrations in the extracellular space facilitating the mechanisms need to maintain glucose homeostasis. GK might be responsible of glucose sensing in some of hypothalamic GE and GI neurons (VMH, LH ARC, PVN and dorsomedial nucleus). GK properties of high-Km phosphorylation of glucose and that it is not inhibited by glucose-6-phosphate, enable that glucose catabolism be proportional to glucose levels in the extracellular space. Also, the functional coexpression of GK with GLUT-2, GKRP and GLP-1R in hypothalamic areas implied in feeding behaviour, could orchestrate regulatory signals to maintain body weight and energy balance. Glucose, orexigenic and anorexigenic peptides contribute to control GK, as well as other energetic sensors such as AMPK. The effect of nutrients occurs on GK and AMPK enzyme activities as required by a short-term response and in a distinctive pattern between VMH and LH. Also, GLP-1 produced a significant reduction of glucose metabolism in selective areas of human brain including hypothalamus and brainstem, both areas involved in feeding behaviour. These findings open new doors for studying the effects of others regulatory peptides in subjects under control and pathophysiological situations. In summary, this chapter has shown a view of the complexity of the network of regulatory signals, where GK, AMPK and regulatory peptides are involved, leading to an optimal energy balance and body weight.

8. Acknowledgment

9. References

Adachi, A.; Kobashi, M. & Funahashi, M. (1995). Glucose-responsive neurons in the brainstem. *Obes Res,* Vol.3 Suppl 5, (Dec), pp. 735S-740S.

Adage, T.; Scheurink, A. J.; de Boer, S. F.; de Vries, K.; Konsman, J. P.; Kuipers, F.; Adan, R. A.; Baskin, D. G.; Schwartz, M. W. & van Dijk, G. (2001). Hypothalamic, metabolic, and behavioral responses to pharmacological inhibition of CNS melanocortin signaling in rats. *J Neurosci,* Vol.21, No.10, (May 15), pp. 3639-3645.

Agius, L. & Peak, M. (1993). Intracellular binding of glucokinase in hepatocytes and translocation by glucose, fructose and insulin. *Biochem J,* Vol.296 (Pt 3), (Dec 15), pp. 785-796.

Alvarez, E.; Martinez, M. D.; Roncero, I.; Chowen, J. A.; Garcia-Cuartero, B.; Gispert, J. D.; Sanz, C.; Vazquez, P.; Maldonado, A.; de Caceres, J.; Desco, M.; Pozo, M. A. & Blazquez, E. (2005). The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *J Neurochem,* Vol.92, No.4, (Feb), pp. 798-806.

Alvarez, E.; Roncero, I.; Chowen, J. A.; Thorens, B. & Blazquez, E. (1996). Expression of the glucagon-like peptide-1 receptor gene in rat brain. *J Neurochem,* Vol.66, No.3, (Mar), pp. 920-927.

Alvarez, E.; Roncero, I.; Chowen, J. A.; Vazquez, P. & Blazquez, E. (2002). Evidence that glucokinase regulatory protein is expressed and interacts with glucokinase in rat brain. *J Neurochem,* Vol.80, No.1, (Jan), pp. 45-53.

Anand, B. K.; Chhina, G. S.; Sharma, K. N.; Dua, S. & Singh, B. (1964). Activity of Single Neurons in the Hypothalamic Feeding Centers: Effect of Glucose. *Am J Physiol,* Vol.207, (Nov), pp. 1146-1154.

Arden, C.; Baltrusch, S. & Agius, L. (2006). Glucokinase regulatory protein is associated with mitochondria in hepatocytes. *FEBS Lett,* Vol.580, No.8, (Apr 3), pp. 2065-2070.

Ashcroft, F. & Rorsman, P. (2004). Type 2 diabetes mellitus: not quite exciting enough? *Hum Mol Genet,* Vol.13 Spec No 1, (Apr 1), pp. R21-31.

Ashford, M. L.; Boden, P. R. & Treherne, J. M. (1990). Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K+ channels. *Pflugers Arch,* Vol.415, No.4, (Jan), pp. 479-483.

Baltrusch, S.; Lenzen, S.; Okar, D. A.; Lange, A. J. & Tiedge, M. (2001). Characterization of glucokinase-binding protein epitopes by a phage-displayed peptide library. Identification of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase as a novel interaction partner. *J Biol Chem,* Vol.276, No.47, (Nov 23), pp. 43915-43923.

Batterham, R. L.; Cowley, M. A.; Small, C. J.; Herzog, H.; Cohen, M. A.; Dakin, C. L.; Wren, A. M.; Brynes, A. E.; Low, M. J.; Ghatei, M. A.; Cone, R. D. & Bloom, S. R. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature,* Vol.418, No.6898, (Aug 8), pp. 650-654.

Bernard, C. (1849). Chiens rendus diabetiquex. *Compt End Soc Biol,* Vol.1, pp. 60

Borg, W. P.; Sherwin, R. S.; During, M. J.; Borg, M. A. & Shulman, G. I. (1995). Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. *Diabetes,* Vol.44, No.2, (Feb), pp. 180-184.

Bray, G. A. (1992). Peptides affect the intake of specific nutrients and the sympathetic nervous system. *Am J Clin Nutr,* Vol.55, No.1 Suppl, (Jan), pp. 265S-271S.

Burdakov, D. & Ashcroft, F. M. (2002). Shedding new light on brain metabolism and glial function. *J Physiol,* Vol.544, No.Pt 2, (Oct 15), pp. 334.

Burdakov, D. & Lesage, F. (2010). Glucose-induced inhibition: how many ionic mechanisms? *Acta Physiol (Oxf),* Vol.198, No.3, (Mar), pp. 295-301.

Burdakov, D.; Luckman, S. M. & Verkhratsky, A. (2005). Glucose-sensing neurons of the hypothalamus. *Philosophical Transactions of the Royal Society B: Biological Sciences,* Vol.360, No.1464, (December 29, 2005), pp. 2227-2235.

Claret, M.; Smith, M. A.; Batterham, R. L.; Selman, C.; Choudhury, A. I.; Fryer, L. G.; Clements, M.; Al-Qassab, H.; Heffron, H.; Xu, A. W.; Speakman, J. R.; Barsh, G. S.; Viollet, B.; Vaulont, S.; Ashford, M. L.; Carling, D. & Withers, D. J. (2007). AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. *J Clin Invest,* Vol.117, No.8, (Aug), pp. 2325-2336.

Coiro, V.; Saccani-Jotti, G.; Rubino, P.; Manfredi, G.; Melani, A. & Chiodera, P. (2006). Effects of ghrelin on circulating neuropeptide Y levels in humans. *Neuro Endocrinol Lett,* Vol.27, No.6, (Dec), pp. 755-757.

Cone, R. D.; Cowley, M. A.; Butler, A. A.; Fan, W.; Marks, D. L. & Low, M. J. (2001). The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *Int J Obes Relat Metab Disord,* Vol.25 Suppl 5, (Dec), pp. S63-67.

Cornish-Bowden, A. & Storer, A. C. (1986). Mechanistic origin of the sigmoidal rate behaviour of rat liver hexokinase D ('glucokinase'). *Biochem J,* Vol.240, No.1, (Nov 15), pp. 293-296.

Cota, D.; Proulx, K.; Smith, K. A.; Kozma, S. C.; Thomas, G.; Woods, S. C. & Seeley, R. J. (2006). Hypothalamic mTOR signaling regulates food intake. *Science,* Vol.312, No.5775, (May 12), pp. 927-930.

Christesen, H. B.; Jacobsen, B. B.; Odili, S.; Buettger, C.; Cuesta-Munoz, A.; Hansen, T.; Brusgaard, K.; Massa, O.; Magnuson, M. A.; Shiota, C.; Matschinsky, F. M. & Barbetti, F. (2002). The second activating glucokinase mutation (A456V): implications for glucose homeostasis and diabetes therapy. *Diabetes,* Vol.51, No.4, (Apr), pp. 1240-1246.

Dalvi, P. S.; Nazarians-Armavil, A.; Tung, S. & Belsham, D. D. (2011). Immortalized Neurons for the Study of Hypothalamic Function. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* Vol., pp.

Dallaporta, M.; Himmi, T.; Perrin, J. & Orsini, J. C. (1999). Solitary tract nucleus sensitivity to moderate changes in glucose level. *Neuroreport,* Vol.10, No.12, (Aug 20), pp. 2657-2660.

de Lecea, L.; Kilduff, T. S.; Peyron, C.; Gao, X.; Foye, P. E.; Danielson, P. E.; Fukuhara, C.; Battenberg, E. L.; Gautvik, V. T.; Bartlett, F. S., 2nd; Frankel, W. N.; van den Pol, A. N.; Bloom, F. E.; Gautvik, K. M. & Sutcliffe, J. G. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A,* Vol.95, No.1, (Jan 6), pp. 322-327.

Diez-Sampedro, A.; Hirayama, B. A.; Osswald, C.; Gorboulev, V.; Baumgarten, K.; Volk, C.; Wright, E. M. & Koepsell, H. (2003). A glucose sensor hiding in a family of transporters. *Proc Natl Acad Sci U S A,* Vol.100, No.20, (Sep 30), pp. 11753-11758.

Drucker, D. J. & Asa, S. (1988). Glucagon gene expression in vertebrate brain. *J Biol Chem,* Vol.263, No.27, (Sep 25), pp. 13475-13478.

Dunn-Meynell, A. A.; Rawson, N. E. & Levin, B. E. (1998). Distribution and phenotype of neurons containing the ATP-sensitive K+ channel in rat brain. *Brain Research,* Vol.814, No.1-2, pp. 41-54.

Dunn-Meynell, A. A.; Routh, V. H.; Kang, L.; Gaspers, L. & Levin, B. E. (2002). Glucokinase is the likely mediator of glucosensing in both glucose-excited and glucose-inhibited central neurons. *Diabetes,* Vol.51, No.7, (Jul), pp. 2056-2065.

Elmquist, J. K.; Elias, C. F. & Saper, C. B. (1999). From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron,* Vol.22, No.2, (Feb), pp. 221-232.

Fioramonti, X.; Lorsignol, A.; Taupignon, A. & Penicaud, L. (2004). A new ATP-sensitive K+ channel-independent mechanism is involved in glucose-excited neurons of mouse arcuate nucleus. *Diabetes,* Vol.53, No.11, (Nov), pp. 2767-2775.

Garcia-Flores, M.; Zueco, J. A.; Arenas, J. & Blazquez, E. (2002). Expression of glucose transporter-2, glucokinase and mitochondrial glycerolphosphate dehydrogenase in pancreatic islets during rat ontogenesis. *Eur J Biochem,* Vol.269, No.1, (Jan), pp. 119-127.

Gonzalez, J. A.; Reimann, F. & Burdakov, D. (2009). Dissociation between sensing and metabolism of glucose in sugar sensing neurones. *J Physiol,* Vol.587, No.Pt 1, (Jan 15), pp. 41-48.

Gribble, F. M.; Williams, L.; Simpson, A. K. & Reimann, F. (2003). A novel glucose-sensing mechanism contributing to glucagon-like peptide-1 secretion from the GLUTag cell line. *Diabetes,* Vol.52, No.5, (May), pp. 1147-1154.

Grupe, A.; Hultgren, B.; Ryan, A.; Ma, Y. H.; Bauer, M. & Stewart, T. A. (1995). Transgenic knockouts reveal a critical requirement for pancreatic beta cell glucokinase in maintaining glucose homeostasis. *Cell,* Vol.83, No.1, (Oct 6), pp. 69-78.

Han, S. M.; Namkoong, C.; Jang, P. G.; Park, I. S.; Hong, S. W.; Katakami, H.; Chun, S.; Kim, S. W.; Park, J. Y.; Lee, K. U. & Kim, M. S. (2005). Hypothalamic AMP-activated protein kinase mediates counter-regulatory responses to hypoglycaemia in rats. *Diabetologia,* Vol.48, No.10, (Oct), pp. 2170-2178.

Hara, J.; Beuckmann, C. T.; Nambu, T.; Willie, J. T.; Chemelli, R. M.; Sinton, C. M.; Sugiyama, F.; Yagami, K.; Goto, K.; Yanagisawa, M. & Sakurai, T. (2001). Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron,* Vol.30, No.2, (May), pp. 345-354.

Hardie, D. G. (2007). AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol,* Vol.8, No.10, (Oct), pp. 774-785.

Hardie, D. G.; Carling, D. & Carlson, M. (1998). The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? *Annu Rev Biochem,* Vol.67, pp. 821-855.

Hardie, D. G.; Hawley, S. A. & Scott, J. W. (2006). AMP-activated protein kinase--development of the energy sensor concept. *J Physiol,* Vol.574, No.Pt 1, (Jul 1), pp. 7-15.

Hughes, S. D.; Quaade, C.; Milburn, J. L.; Cassidy, L. & Newgard, C. B. (1991). Expression of normal and novel glucokinase mRNAs in anterior pituitary and islet cells. *J Biol Chem,* Vol.266, No.7, (Mar 5), pp. 4521-4530.

Iynedjian, P. B. (1993). Mammalian glucokinase and its gene. *Biochem J,* Vol.293 (Pt 1), (Jul 1), pp. 1-13.

Iynedjian, P. B.; Marie, S.; Wang, H.; Gjinovci, A. & Nazaryan, K. (1996). Liver-specific enhancer of the glucokinase gene. *J Biol Chem,* Vol.271, No.46, (Nov 15), pp. 29113-29120.

Jordan, S. D.; Konner, A. C. & Bruning, J. C. (2010). Sensing the fuels: glucose and lipid signaling in the CNS controlling energy homeostasis. *Cell Mol Life Sci,* Vol.67, No.19, (Oct), pp. 3255-3273.

Kang, L.; Dunn-Meynell, A. A.; Routh, V. H.; Gaspers, L. D.; Nagata, Y.; Nishimura, T.; Eiki, J.; Zhang, B. B. & Levin, B. E. (2006). Glucokinase is a critical regulator of ventromedial hypothalamic neuronal glucosensing. *Diabetes,* Vol.55, No.2, (Feb), pp. 412-420.

Kang, L.; Routh, V. H.; Kuzhikandathil, E. V.; Gaspers, L. D. & Levin, B. E. (2004). Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. *Diabetes,* Vol.53, No.3, (Mar), pp. 549-559.

Kola, B.; Hubina, E.; Tucci, S. A.; Kirkham, T. C.; Garcia, E. A.; Mitchell, S. E.; Williams, L. M.; Hawley, S. A.; Hardie, D. G.; Grossman, A. B. & Korbonits, M. (2005). Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *J Biol Chem,* Vol.280, No.26, (Jul 1), pp. 25196-25201.

Kristensen, B. W.; Noer, H.; Gramsbergen, J. B.; Zimmer, J. & Noraberg, J. (2003). Colchicine induces apoptosis in organotypic hippocampal slice cultures. *Brain Res,* Vol.964, No.2, (Feb 28), pp. 264-278.

Lee, K.; Li, B.; Xi, X.; Suh, Y. & Martin, R. J. (2005). Role of neuronal energy status in the regulation of adenosine 5'-monophosphate-activated protein kinase, orexigenic neuropeptides expression, and feeding behavior. *Endocrinology,* Vol.146, No.1, (Jan), pp. 3-10.

Leibowitz, S. F. (1992). Neurochemical-neuroendocrine systems in the brain controlling macronutrient intake and metabolism. *Trends Neurosci,* Vol.15, No.12, (Dec), pp. 491-497.

Levin, B. E.; Routh, V. H.; Kang, L.; Sanders, N. M. & Dunn-Meynell, A. A. (2004). Neuronal glucosensing: what do we know after 50 years? *Diabetes,* Vol.53, No.10, (Oct), pp. 2521-2528.

Lossi, L.; Alasia, S.; Salio, C. & Merighi, A. (2009). Cell death and proliferation in acute slices and organotypic cultures of mammalian CNS. *Prog Neurobiol,* Vol.88, No.4, (Aug), pp. 221-245.

Lynch, R. M.; Tompkins, L. S.; Brooks, H. L.; Dunn-Meynell, A. A. & Levin, B. E. (2000). Localization of glucokinase gene expression in the rat brain. *Diabetes,* Vol.49, No.5, (May 1, 2000), pp. 693-700.

Magnuson, M. A. & Shelton, K. D. (1989). An alternate promoter in the glucokinase gene is active in the pancreatic beta cell. *J Biol Chem,* Vol.264, No.27, (Sep 25), pp. 15936-15942.

Malaisse, W. J.; Malaisse-Lagae, F.; Davies, D. R.; Vandercammen, A. & Van Schaftingen, E. (1990). Regulation of glucokinase by a fructose-1-phosphate-sensitive protein in pancreatic islets. *Eur J Biochem,* Vol.190, No.3, (Jul 5), pp. 539-545.

Matschinsky, F.; Liang, Y.; Kesavan, P.; Wang, L.; Froguel, P.; Velho, G.; Cohen, D.; Permutt, M. A.; Tanizawa, Y.; Jetton, T. L. & et al. (1993). Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. *J Clin Invest,* Vol.92, No.5, (Nov), pp. 2092-2098.

Matschinsky, F. M. (1990). Glucokinase as glucose sensor and metabolic signal generator in pancreatic beta-cells and hepatocytes. *Diabetes,* Vol.39, No.6, (Jun), pp. 647-652.

Mayer, J. (1953). Glucostatic Mechanism of Regulation of Food Intake. *New England Journal of Medicine,* Vol.249, No.1, pp. 13-16.

McCrimmon, R. J.; Fan, X.; Cheng, H.; McNay, E.; Chan, O.; Shaw, M.; Ding, Y.; Zhu, W. & Sherwin, R. S. (2006). Activation of AMP-activated protein kinase within the ventromedial hypothalamus amplifies counterregulatory hormone responses in rats with defective counterregulation. *Diabetes,* Vol.55, No.6, (Jun), pp. 1755-1760.

Minokoshi, Y.; Alquier, T.; Furukawa, N.; Kim, Y. B.; Lee, A.; Xue, B.; Mu, J.; Foufelle, F.; Ferre, P.; Birnbaum, M. J.; Stuck, B. J. & Kahn, B. B. (2004). AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature,* Vol.428, No.6982, (Apr 1), pp. 569-574.

Mora, F.; Exposito, I.; Sanz, B. & Blazquez, E. (1992). Selective release of glutamine and glutamic acid produced by perfusion of GLP-1 (7-36) amide in the basal ganglia of the conscious rat. *Brain Res Bull,* Vol.29, No.3-4, (Sep-Oct), pp. 359-361.

Morrison, B., 3rd; Cater, H. L.; Benham, C. D. & Sundstrom, L. E. (2006). An in vitro model of traumatic brain injury utilising two-dimensional stretch of organotypic hippocampal slice cultures. *J Neurosci Methods,* Vol.150, No.2, (Jan 30), pp. 192-201.

Moukil, M. A.; Veiga-da-Cunha, M. & Van Schaftingen, E. (2000). Study of the regulatory properties of glucokinase by site-directed mutagenesis: conversion of glucokinase to an enzyme with high affinity for glucose. *Diabetes,* Vol.49, No.2, (Feb), pp. 195-201.

Mountjoy, P. D. & Rutter, G. A. (2007). Glucose sensing by hypothalamic neurones and pancreatic islet cells: AMPle evidence for common mechanisms? *Exp Physiol,* Vol.92, No.2, (Mar), pp. 311-319.

Munoz-Alonso, M. J.; Guillemain, G.; Kassis, N.; Girard, J.; Burnol, A. F. & Leturque, A. (2000). A novel cytosolic dual specificity phosphatase, interacting with glucokinase, increases glucose phosphorylation rate. *J Biol Chem,* Vol.275, No.42, (Oct 20), pp. 32406-32412.

Navarro, M.; Rodriquez de Fonseca, F.; Alvarez, E.; Chowen, J. A.; Zueco, J. A.; Gomez, R.; Eng, J. & Blazquez, E. (1996). 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,* Vol.67, No.5, (Nov), pp. 1982-1991.

Niswender, K. (2010). Diabetes and obesity: therapeutic targeting and risk reduction - a complex interplay. *Diabetes Obes Metab,* Vol.12, No.4, (Apr), pp. 267-287.

Noraberg, J.; Poulsen, F. R.; Blaabjerg, M.; Kristensen, B. W.; Bonde, C.; Montero, M.; Meyer, M.; Gramsbergen, J. B. & Zimmer, J. (2005). Organotypic hippocampal slice cultures
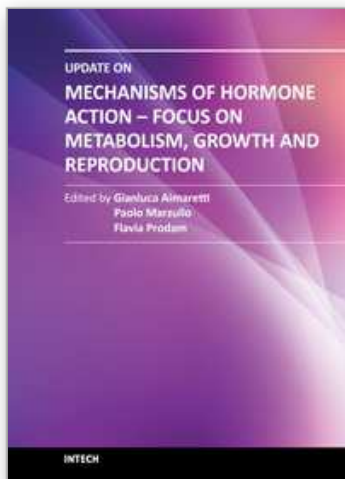
for studies of brain damage, neuroprotection and neurorepair. *Curr Drug Targets CNS Neurol Disord,* Vol.4, No.4, (Aug), pp. 435-452.

Oomura, Y.; Ono, T.; Ooyama, H. & Wayner, M. J. (1969). Glucose and osmosensitive neurones of the rat hypothalamus. *Nature,* Vol.222, No.5190, (Apr 19), pp. 282-284.

Oomura, Y.; Ooyama, H.; Sugimori, M.; Nakamura, T. & Yamada, Y. (1974). Glucose inhibition of the glucose-sensitive neurone in the rat lateral hypothalamus. *Nature,* Vol.247, No.439, (Feb 1), pp. 284-286.

Oomura, Y. & Yoshimatsu, H. (1984). Neural network of glucose monitoring system. *J Auton Nerv Syst,* Vol.10, No.3-4, (May-Jun), pp. 359-372.

Orskov, C.; Poulsen, S. S.; Moller, M. & Holst, J. J. (1996). Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. *Diabetes,* Vol.45, No.6, (Jun), pp. 832-835.

Pellerin, L. & Magistretti, P. J. (2004). Neuroenergetics: calling upon astrocytes to satisfy hungry neurons. *Neuroscientist,* Vol.10, No.1, (Feb), pp. 53-62.

Penicaud, L.; Leloup, C.; Fioramonti, X.; Lorsignol, A. & Benani, A. (2006). Brain glucose sensing: a subtle mechanism. *Curr Opin Clin Nutr Metab Care,* Vol.9, No.4, (Jul), pp. 458-462.

Perrin, C.; Knauf, C. & Burcelin, R. (2004). Intracerebroventricular infusion of glucose, insulin, and the adenosine monophosphate-activated kinase activator, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, controls muscle glycogen synthesis. *Endocrinology,* Vol.145, No.9, (Sep), pp. 4025-4033.

Ritter, S. & Dinh, T. T. (1994). 2-Mercaptoacetate and 2-deoxy-D-glucose induce Fos-like immunoreactivity in rat brain. *Brain Res,* Vol.641, No.1, (Mar 28), pp. 111-120.

Ritter, S.; Dinh, T. T. & Zhang, Y. (2000). Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain Res,* Vol.856, No.1-2, (Feb 21), pp. 37-47.

Rodriguez de Fonseca, F.; Navarro, M.; Alvarez, E.; Roncero, I.; Chowen, J. A.; Maestre, O.; Gomez, R.; Munoz, R. M.; Eng, J. & Blazquez, E. (2000). Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats. *Metabolism,* Vol.49, No.6, (Jun), pp. 709-717.

Roncero, I.; Alvarez, E.; Chowen, J. A.; Sanz, C.; Rabano, A.; Vazquez, P. & Blazquez, E. (2004). Expression of glucose transporter isoform GLUT-2 and glucokinase genes in human brain. *J Neurochem,* Vol.88, No.5, (Mar), pp. 1203-1210.

Roncero, I.; Alvarez, E.; Vazquez, P. & Blazquez, E. (2000). Functional glucokinase isoforms are expressed in rat brain. *J Neurochem,* Vol.74, No.5, (May), pp. 1848-1857.

Roncero, I.; Sanz, C.; Alvarez, E.; Vazquez, P.; Barrio, P. A. & Blazquez, E. (2009). Glucokinase and glucokinase regulatory proteins are functionally coexpressed before birth in the rat brain. *J Neuroendocrinol,* Vol.21, No.12, (Dec), pp. 973-981.

Ropelle, E. R.; Pauli, J. R.; Fernandes, M. F.; Rocco, S. A.; Marin, R. M.; Morari, J.; Souza, K. K.; Dias, M. M.; Gomes-Marcondes, M. C.; Gontijo, J. A.; Franchini, K. G.; Velloso, L. A.; Saad, M. J. & Carvalheira, J. B. (2008). A central role for neuronal AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) in high-protein diet-induced weight loss. *Diabetes,* Vol.57, No.3, (Mar), pp. 594-605.

Routh, V. H. (2002). Glucose-sensing neurons: Are they physiologically relevant? *Physiology & Behavior,* Vol.76, No.3, pp. 403-413.

Rutter, G. A.; Da Silva Xavier, G. & Leclerc, I. (2003). Roles of 5'-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. *Biochem J,* Vol.375, No.Pt 1, (Oct 1), pp. 1-16.

Sakurai, T.; Amemiya, A.; Ishii, M.; Matsuzaki, I.; Chemelli, R. M.; Tanaka, H.; Williams, S. C.; Richardson, J. A.; Kozlowski, G. P.; Wilson, S.; Arch, J. R.; Buckingham, R. E.; Haynes, A. C.; Carr, S. A.; Annan, R. S.; McNulty, D. E.; Liu, W. S.; Terrett, J. A.; Elshourbagy, N. A.; Bergsma, D. J. & Yanagisawa, M. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell,* Vol.92, No.4, (Feb 20), pp. 573-585.

Sanz, C.; Roncero, I.; Vazquez, P.; Navas, M. A. & Blazquez, E. (2007). Effects of glucose and insulin on glucokinase activity in rat hypothalamus. *J Endocrinol,* Vol.193, No.2, (May), pp. 259-267.

Sanz, C.; Vazquez, P.; Navas, M. A.; Alvarez, E. & Blazquez, E. (2008). Leptin but not neuropeptide Y up-regulated glucagon-like peptide 1 receptor expression in GT1-7 cells and rat hypothalamic slices. *Metabolism,* Vol.57, No.1, (Jan), pp. 40-48.

Schuit, F. C.; Huypens, P.; Heimberg, H. & Pipeleers, D. G. (2001). Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. *Diabetes,* Vol.50, No.1, (Jan), pp. 1-11.

Shiota, C.; Coffey, J.; Grimsby, J.; Grippo, J. F. & Magnuson, M. A. (1999). Nuclear import of hepatic glucokinase depends upon glucokinase regulatory protein, whereas export is due to a nuclear export signal sequence in glucokinase. *J Biol Chem,* Vol.274, No.52, (Dec 24), pp. 37125-37130.

Silver, I. A. & Erecinska, M. (1994). 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,* Vol.14, No.8, (Aug), pp. 5068-5076.

Silver, I. A. & Erecinska, M. (1998). Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. *J Neurophysiol,* Vol.79, No.4, (Apr), pp. 1733-1745.

Song, Z. & Routh, V. H. (2005). Differential effects of glucose and lactate on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes,* Vol.54, No.1, (Jan), pp. 15-22.

Sundstrom, L.; Morrison, B., 3rd; Bradley, M. & Pringle, A. (2005). Organotypic cultures as tools for functional screening in the CNS. *Drug Discov Today,* Vol.10, No.14, (Jul 15), pp. 993-1000.

Sutherland, V. L.; McReynolds, M.; Tompkins, L. S.; Brooks, H. L. & Lynch, R. M. (2005). Developmental expression of glucokinase in rat hypothalamus. *Brain Res Dev Brain Res,* Vol.154, No.2, (Feb 8), pp. 255-258.

Tang-Christensen, M.; Larsen, P. J.; Thulesen, J.; Romer, J. & Vrang, N. (2000). The proglucagon-derived peptide, glucagon-like peptide-2, is a neurotransmitter involved in the regulation of food intake. *Nat Med,* Vol.6, No.7, (Jul), pp. 802-807.

Tippett, P. S. & Neet, K. E. (1982). An allosteric model for the inhibition of glucokinase by long chain acyl coenzyme A. *J Biol Chem*, Vol.257, No.21, (Nov 10), pp. 12846-12852.

Tong, Q.; Ye, C.; McCrimmon, R. J.; Dhillon, H.; Choi, B.; Kramer, M. D.; Yu, J.; Yang, Z.; Christiansen, L. M.; Lee, C. E.; Choi, C. S.; Zigman, J. M.; Shulman, G. I.; Sherwin, R. S.; Elmquist, J. K. & Lowell, B. B. (2007). Synaptic glutamate release by ventromedial hypothalamic neurons is part of the neurocircuitry that prevents hypoglycemia. *Cell Metab*, Vol.5, No.5, (May), pp. 383-393.

Toyoda, Y.; Miwa, I.; Satake, S.; Anai, M. & Oka, Y. (1995). Nuclear location of the regulatory protein of glucokinase in rat liver and translocation of the regulator to the cytoplasm in response to high glucose. *Biochem Biophys Res Commun*, Vol.215, No.2, (Oct 13), pp. 467-473.

Tsuneki, H.; Wada, T. & Sasaoka, T. (2010). Role of orexin in the regulation of glucose homeostasis. *Acta Physiol (Oxf)*, Vol.198, No.3, (Mar), pp. 335-348.

Turnley, A. M.; Stapleton, D.; Mann, R. J.; Witters, L. A.; Kemp, B. E. & Bartlett, P. F. (1999). Cellular distribution and developmental expression of AMP-activated protein kinase isoforms in mouse central nervous system. *J Neurochem*, Vol.72, No.4, (Apr), pp. 1707-1716.

Turton, M. D.; O'Shea, D.; Gunn, I.; Beak, S. A.; Edwards, C. M.; Meeran, K.; Choi, S. J.; Taylor, G. M.; Heath, M. M.; Lambert, P. D.; Wilding, J. P.; Smith, D. M.; Ghatei, M. A.; Herbert, J. & Bloom, S. R. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature*, Vol.379, No.6560, (Jan 4), pp. 69-72.

Uttenthal, L. O.; Toledano, A. & Blazquez, E. (1992). Autoradiographic localization of receptors for glucagon-like peptide-1 (7-36) amide in rat brain. *Neuropeptides*, Vol.21, No.3, (Mar), pp. 143-146.

Van Schaftingen, E. (1989). A protein from rat liver confers to glucokinase the property of being antagonistically regulated by fructose 6-phosphate and fructose 1-phosphate. *Eur J Biochem*, Vol.179, No.1, (Jan 15), pp. 179-184.

Van Schaftingen, E.; Bartrons, R. & Hers, H. G. (1984). The mechanism by which ethanol decreases the concentration of fructose 2,6-bisphosphate in the liver. *Biochem J*, Vol.222, No.2, (Sep 1), pp. 511-518.

Vandercammen, A. & Van Schaftingen, E. (1993). Species and tissue distribution of the regulatory protein of glucokinase. *Biochem J*, Vol.294 (Pt 2), (Sep 1), pp. 551-556.

Viollet, B.; Athea, Y.; Mounier, R.; Guigas, B.; Zarrinpashneh, E.; Horman, S.; Lantier, L.; Hebrard, S.; Devin-Leclerc, J.; Beauloye, C.; Foretz, M.; Andreelli, F.; Ventura-Clapier, R. & Bertrand, L. (2009). AMPK: Lessons from transgenic and knockout animals. *Front Biosci*, Vol.14, pp. 19-44.

Vionnet, N.; Stoffel, M.; Takeda, J.; Yasuda, K.; Bell, G. I.; Zouali, H.; Lesage, S.; Velho, G.; Iris, F.; Passa, P. & et al. (1992). Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature*, Vol.356, No.6371, (Apr 23), pp. 721-722.

Wang, R.; Liu, X.; Hentges, S. T.; Dunn-Meynell, A. A.; Levin, B. E.; Wang, W. & Routh, V. H. (2004). The regulation of glucose-excited neurons in the hypothalamic arcuate

nucleus by glucose and feeding-relevant peptides. *Diabetes,* Vol.53, No.8, (Aug), pp. 1959-1965.

Yang, X. J.; Kow, L. M.; Funabashi, T. & Mobbs, C. V. (1999). Hypothalamic glucose sensor: similarities to and differences from pancreatic beta-cell mechanisms. *Diabetes,* Vol.48, No.9, (Sep), pp. 1763-1772.

Yang, X. J.; Kow, L. M.; Pfaff, D. W. & Mobbs, C. V. (2004). Metabolic pathways that mediate inhibition of hypothalamic neurons by glucose. *Diabetes,* Vol.53, No.1, (Jan), pp. 67-73.

Zhou, L.; Podolsky, N.; Sang, Z.; Ding, Y.; Fan, X.; Tong, Q.; Levin, B. E. & McCrimmon, R. J. (2010). The Medial Amygdalar Nucleus: A Novel Glucose-Sensing Region That Modulates the Counterregulatory Response to Hypoglycemia. *Diabetes,* Vol.59, No.10, (October 1, 2010), pp. 2646-2652.

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Edited by Prof. Gianluca Aimaretti

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