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Diepenbroek, C.

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Chapter 1

General introduction - Part 1
Brain areas and pathways in the regulation of glucose metabolism

Charlene Diepenbroek, Mireille J. Serlie, Eric Fliers, Andries Kalsbeek, Susanne E. la Fleur

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Chapter 1

Introduction
The central nervous system (CNS) derives its energy mainly from blood glucose. Therefore, in order for an organism to function, it is essential to maintain circulating glucose concentrations at a sufficiently high level. The brain, therefore ensures that blood glucose concentrations are maintained between physiological ranges, depending on the species, between 3.0 and 5.6mM (euglycaemia) while the glucose concentration within the brain is buffered at a much lower concentration and within a narrower physiological range (0.5–2.5mM) (Watts & Donovan, 2010).

Following glycaemic challenges, humoral and neural signals will act as regulatory responses to balance glucose production (mainly by the liver) and glucose uptake (mainly by muscle, adipose and brain tissue) eventually restoring euglycaemia. For example, when systemic glucose concentrations increase after food ingestion, glucose uptake has to be stimulated and glucose production has to be suppressed. Therefore parasympathetic efferents, mainly those projecting to the liver and the endocrine pancreas will be activated. Parasympathetic innervation of the liver stimulates conversion of glucose to glycogen (Shimazu, 1998), while parasympathetic innervation of the endocrine pancreas stimulates insulin secretion from the β-cells. Insulin inhibits hepatic glucose production and stimulates glucose disposal in insulin sensitive tissues restoring euglycaemia. However, also prior to elevations in blood glucose concentrations, insulin may be released. This so-called cephalic phase reflex is already induced upon activation of taste receptors (and subsequently their afferent projections) in the oral cavity (Teff & Townsend, 1999). In addition, macronutrients in the intestine trigger duodenal K- and ileal L- cells to secrete the incretins glucose-induced insulinoctropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), respectively. Both hormones will stimulate insulin secretion (for review on central and peripheral action of GLP-1 see (Sandoval, 2008)). Besides its direct effects on glucose metabolism, insulin also suppresses the secretion of glucagon by the α-cells of the pancreas and thereby also affects hepatic glucose production indirectly.

In fasting conditions, a decrease in plasma glucose concentrations triggers a counter-regulatory response consisting of an increase in glucagon, adrenaline, corticosterone and growth hormone secretion while insulin concentrations are low. As reviewed by Beall et al. (2012) glucose thresholds for counter-regulatory responses in humans are around 4.4mM for glucagon, <3.8mM for adrenaline and noradrenaline and <3.7mM for corticosterone and growth hormone. Glucagon secretion is stimulated during hypoglycaemia and stimulates hepatic glucose production. Noradrenaline, released from sympathetic nerve endings, and adrenaline, released from the adrenal glands, stimulate lipolysis, glycogenolysis, decrease insulin secretion and reduce insulin sensitivity. Gluconeogenesis (de novo production of glucose from non-glucose precursors like amino acids), is stimulated by corticosterone and growth hormone. The latter also stimulate lipolysis and ketogenesis. Lipolysis yields fatty acids and glycerol, which are
used for substrate oxidation and gluconeogenesis respectively. Counter-regulation of hypoglycaemia by corticosterone and growth hormone is considered as an adaptive response (Beall et al., 2012; Watts & Donovan, 2010). These multiple mechanisms by which the brain ensures sufficiently high blood glucose concentrations are controlled by many regions in the brain. It has long been recognized (and extensively reviewed) how the brain stem and hypothalamus are key players in the control of glucose metabolism, as many glucose sensing neurons reside in these areas (Marty et al., 2007). Interestingly, the hypothalamus receives strong inputs from corticolimbic brain areas as well. As also many of the limbic structures contain glucose sensing neurons and have strong connections with hypothalamic areas this review here focuses on the involvement of the hypothalamus and corticolimbic areas in regulating glucose metabolism.

Hypothalamic areas in the control of glucose metabolism

The preferred nutrient used by the brain is glucose (Belanger et al., 2011). It is therefore not surprising that the brain responds to changes in plasma glucose concentrations. The brain can regulate glucose production and uptake via either stimulating hormone release through activating hypothalamus pituitary hormonal axes (for example releasing corticosterone), but interestingly, anatomical tracing experiments revealed that there are also neural connections between the hypothalamus and the liver, pancreas and adipose tissue via the autonomic nervous system (ANS) (Kalsbeek et al., 2010) involved in regulating glucose concentrations.

The brain has several ways to sense whether circulating glucose concentrations need to be adjusted. Throughout the hypothalamus, several populations of glucose-sensing neurons, which are either glucose excited (GE) or glucose inhibited (GI) when extracellular glucose concentrations change, reside (for review on hypothalamic glucose sensing neurons see Burdakov & Gonzalez (2009); Levin et al. (2004). Besides glucose sensing neurons, more recently the energy sensing enzyme AMP-activated protein kinase (AMPK) has been proposed as a hypothalamic glucose and nutrient sensor (Han et al., 2005; Kahn et al., 2005; Murphy et al., 2009). AMPK is activated when intracellular energy levels drop and is also involved in the counter-regulatory response to hypoglycaemia (Han et al., 2005). Moreover, hormones like leptin and insulin may act directly within the hypothalamus and brain stem to influence glucose metabolism. For example, in rats and mice insulin administration in the dorsal vagal complex or in the mediobasal hypothalamus lowers glucose production, independent of changes in circulating plasma concentrations of insulin and glucagon (Filippi et al., 2012; Obici et al., 2002b). Within the hypothalamus several nuclei and several neuropeptides are involved in glucose regulation.
Arcuate nucleus (ARC)

Since the discovery of leptin in 1994 (Zhang et al., 1994), much research has been focused on the ARC. The ventral part of the ARC is in close contact with the systemic circulation due to its “leaky” blood-brain-barrier (Ciofi, 2011; Schaeffer et al., 2013) and through this close contact nutrients and hormones can easily influence these neurons. Two main populations of neurons reside within the ARC. On the one hand, there are neurons expressing both neuropeptide Y (NPY) and Agouti Related protein (AGPR) and on the other hand there are neurons that express pro-opiomelanocortins (POMC) and cocaine-amphetamine regulated transcript (CART).

The orexigenic NPY-containing neurons of the ARC, with their projections to several hypothalamic brain areas including the paraventricular nucleus of the hypothalamus (PVN), have been studied extensively. Both rats and mice show increased endogenous glucose production (EGP) following intracerebroventricular administration of NPY (Marks & Waite, 1997). Central administration of NPY, combined with the euglycaemic hyperinsulinaemic clamp technique, which is the gold standard to measure insulin sensitivity, showed that the inhibitory effects of peripheral hyperinsulinemia on hepatic glucose production were (partially) blocked by intracerebroventricular administration of NPY, thereby inducing hepatic insulin resistance. Moreover, specific denervation of hepatic sympathetic nerves blocked the inhibitory effect of NPY on hepatic insulin sensitivity, suggesting that intracerebroventricular NPY acts through the sympathetic innervation on liver glucose metabolism (van den Hoek et al., 2008).

Because of abundant expression of insulin receptors on NPY neurons the brain-mediated inhibitory effect of insulin on hepatic glucose production might in part be induced via the inhibition of neuronal activity of NPY in the ARC. Subsequently, the resulting diminished release of NPY will decrease the stimulatory input to the sympathetic pre-autonomic neurons in the PVN and thus reduce sympathetic stimulation of hepatic glucose production. However, Pocai et al. (2005) showed that also the parasympathetic innervation of the liver is involved in the inhibitory effect of insulin on hepatic glucose production. Moreover, the effects of NPY seem to be specific for glucose production as in none of the above experiments there was a significant effect on whole body glucose uptake.

This means that in addition to the effect of NPY on the sympathetic pre-autonomic neurons another neurotransmitter must be responsible for the transmission of insulin’s effects in the ARC to the parasympathetic pre-autonomic neurons in the PVN. One of the candidates playing this role might be α-MSH, the neurotransmitter of the anorexigenic proopiomelanocortin (POMC) neurons. Central administration of MTII, an α-MSH agonist, dose-dependently inhibited basal insulin secretion (Banno et al., 2007). In addition, the effect of insulin on glucose uptake and production is enhanced by intraventricular administration of α-MSH or MTII and central as well as whole body overexpression of α-MSH improves glucose metabolism pointing to increased insulin
sensitivity (cited in Lee et al., 2007). A relation between α-MSH and insulin sensitivity has been observed in post mortem brains of humans. Recently, Alkemade et al. (2012) showed reduced α-MSH immunoreactivity in the infundibular nucleus, the human equivalent of the ARC, in post mortem brain slices of individuals with a history of type 2 diabetes.

Contradicting these studies, acute central administration of α-MSH increased glucose production through increasing gluconeogenic enzymes (G6P and PEPCK) and this effect could be antagonized by SHU9119 (Gutierrez-Juarez et al., 2004). Of note, a very high dose of α-MSH (3µg) was used in this study questioning the physiological relevance. Surprisingly, intracerebroventricular co-administration of a melanocortin antagonist failed to block the decrease in EGP induced by hyperinsulinemia indicating that α-MSH does not seem to be involved in the inhibitory effect of hypothalamic insulin on EGP (Obici et al., 2002b).

In summary, NPY from the ARC increases EGP and decreases hepatic insulin sensitivity, which is mediated via the sympathetic nervous system. Besides the sympathetic nervous system, the parasympathetic nervous system is involved in the ARC mediated effects on glucose metabolism, via POMC neurons and their neurotransmitter α-MSH. In contrast to NPY, α-MSH in the ARC also affects peripheral glucose disposal.

**Lateral hypothalamic area (LHA)**

The LHA, amongst others, expresses the orexigenic neuropeptides orexin and melanin-concentrating hormone (MCH), which both have been shown to play a role in glucose metabolism. It was shown that central infusion of orexin increases plasma glucose concentrations through an increase in hepatic glucose production, which could be blocked by a sympathetic but not parasympathetic denervation of the liver (Yi et al., 2009). The same effect on glucose metabolism was observed by local activation of orexin neurons via blocking of their GABA inhibition using bicuculline and showing that increased availability of orexin in the CNS, affects plasma glucose concentrations (Yi et al., 2010). In addition, in mice and rats, injection of orexin-A in the VMH promoted glucose uptake and glycogen synthesis in skeletal muscle via activation of the sympathetic nervous system (Shiuchi et al., 2009).

Also expressed in the LHA is MCH, a cyclic 19-amino-acid polypeptide. Orexin and MCH do not co-localize, although they almost completely overlap in distribution. Overexpression of MCH in the LHA resulted in hyperglycemia and insulin resistance (Ludwig et al., 2001). This might be due to increased corticosterone concentrations, observed in that study, as more recent experiments showed that glucose metabolism was not affected in MCH knock-out rats (Mul et al., 2010) nor after intracerebroventricular administered MCH in wild type rats (Yi et al., 2009). In fact, the reduced metabolic rate
found in the MCH knock-out rats was perfectly adapted to the leaner body composition of these MCH knock-out animals. Of course, these data do not rule out a role for MCH in glucose metabolism, but at present its role remains unclear.

**Ventromedial hypothalamus (VMH)**

The VMH plays an important role in the recovery from hypoglycaemia (Borg *et al*., 1994). A prominent population of neurons in the VMH contain pituitary adenylate cyclase activating peptide (PACAP), a 38-amino acid, C-terminally α-amidated neuropeptide. Studies using PACAP knock-outs clearly indicate a role for PACAP in glucose metabolism (for review see Nakata & Yada, 2007). However, these studies did not reveal which component of the metabolic phenotype (leaner compared to wild type littermates due to decreased adiposity (Adams *et al*., 2008) could be attributed to central versus peripheral signalling pathways of PACAP, although some evidence for central effects on energy metabolism is available. Amongst others it has been shown that central administration of PACAP decreases food intake (Mizuno *et al*., 2003; Morley *et al*., 1992) and increases plasma glucose concentrations (Mounien *et al*., 2009). More recently, it was shown that intracerebroventricular administered PACAP increased EGP (Yi *et al*., 2010). Additional tracing and denervation experiments provided strong evidence that the effects of PACAP are mediated through the pre-autonomic neurons in the hypothalamus. Moreover, intracerebroventricular administration of PACAP increased whole body sympathetic nerve activity, whereas parasympathetic nerve activity was decreased (Tanida *et al*., 2010). Thus, the PACAP neurons in the VMH (and ARC) could be an important gateway to control hepatic glucose production during hypoglycaemia.

The role of the VMH in hypoglycemia is also supported by the presence of AMPK. As mentioned before, this enzyme acts as a hypothalamic glucose sensor and is activated during peripheral (Han *et al*., 2005) as well as central hypoglycemia (Alquier *et al*., 2007) while its expression is inhibited during central and peripheral hyperglycaemia (Kim *et al*., 2004; Minokoshi *et al*., 2004). Activation of AMPK in the VMH is associated with counter-regulatory responses to restore euglycaemia. Central administration of 2-deoxy-D-glucose (2-DG), (a glycolysis inhibitor) increased plasma concentrations of glucose, glucagon and corticosterone and activated AMPK in the VMH, dorsomedial hypothalamus (DMH) and ARC. Repeated central administration of 2-DG dampened this counter-regulatory response, which could be partially augmented by central administration of the pharmacological AMPK activator 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR). The same effect of AICAR administration has been observed during recurrent insulin induced hypoglycaemia (McCrimmon *et al*., 2006) suggesting that hypoglycemia unawareness in recurrent hypoglycemia might, in part, be mediated through this pathway. Together these studies support a role for the
VMH in restoring hypoglycemia via AMPK. AMPK activation is suggested to activate phosphorylation of neuronal nitric oxide (NO) synthase, which increases NO production. Binding of NO to its receptor, soluble guanylylcyclase, induces production of guanosine 3’, 5’-cyclic monophosphate. This increases AMPK activation and inactivates the Cl⁻ channel, causing depolarization of the VMH GI neurons (Murphy et al., 2009).

Besides PACAP and AMPK, additional changes in VMH neurotransmitter activity are associated with alterations in glucose metabolism. Local VMH administration of the γ-aminobutyric acid (GABA) agonist muscimol suppressed, while administration of the antagonist bicuculline (BIC) amplified, the magnitude of glucagon and epinephrine responses to insulin induced hypoglycaemia (Chan et al., 2006). Research from the same group previously showed that in vivo manipulation of ATP-sensitive potassium (K_{ATP}) channel activity in the VMH with glibenclamide (which causes depolarization of the neuron) or diazoxide (which causes hyperpolarization of the neuron) respectively suppresses or enhances the counter-regulatory systemic hormonal responses to systemic insulin-induced hypoglycaemia respectively (Evans et al., 2004; McCrimmon et al., 2005). Subsequent studies showed that K_{ATP} channels can modulate GABA release in the VMH (Chan et al., 2007) and that glucose induced increased GABA release can be inhibited by the K_{ATP} channel opener, diazoxide (Zhu et al., 2010).

The role of the neurotransmitters noradrenalin and serotonin in glucose metabolism is especially evident from studies in animals that undergo seasonal changes in which they transit between lean, insulin sensitive and obese, insulin-resistant states (see for review DeFronzo (2011). In hamsters, increased activity of noradrenaline and serotonin in the VMH was associated with an insulin resistant, glucose intolerant state, which could be normalized by local reduction of the activity of these monoamines. Long term, central infusion of noradrenaline and serotonin induces hyperinsulinemia and glucose intolerance without hyperphagia or body weight gain. The hyperinsulinemia might be potentiated by dysregulation of insulin secretion by the pancreatic β-cell. In female rats chronic infusion of noradrenaline in the VMH resulted in glucose intolerance. This effect is specific for the VMH as long term infusion of noradrenaline and serotonin just outside the VMH in hamsters or noradrenaline infusion in the LHA or PVN in rats does not cause hyperinsulinemia (Shimazu et al., 1986). Short-term central administration of noradrenaline to the VMH of insulin sensitive rats causes a rapid increase in plasma concentrations of glucose, insulin and glucagon (Steffens et al., 1984) which can be potentiated by centrally administered serotonin (Suzuki et al., 1995).

To summarize, the VMH is important in restoring euglycaemia following hypoglycemic challenges. PACAP neurons are important in the control of EGP during hypoglycemia and AMPK is (at least partly) involved in activation of counter-regulatory responses. Next to PACAP and AMPK, changes in neurotransmitter concentrations in the VMH
are associated with alterations in glucose metabolism, though modulating glucose tolerance and insulin sensitivity.

**Suprachiasmatic nucleus (SCN)**

Blood glucose concentrations show a daily rhythm in both human and rodents, which is regulated by the suprachiasmatic nucleus (SCN). This nucleus in the hypothalamus is responsible for all daily rhythms, including the sleep-wake cycle, feeding rhythms and endocrine rhythms. Just prior to awakening, humans show higher glucose output while patients with diabetes require more insulin, known as the dawn phenomenon (Bolli et al., 1984; Trumper et al., 1995). In the afternoon glucose tolerance is decreased, due to a decrease in insulin sensitivity and insulin response to glucose administration (Carroll & Nestel, 1973; Lee et al., 1992; Whichelow et al., 1974). Rats show a 12-hour shift in this daily rhythm and show the highest glucose tolerance and insulin sensitivity in the afternoon (la Fleur et al., 2001). These changes in glucose tolerance are suggested to be anticipatory for the following activity period, but it remains unclear which tissues are responsible.

The involvement of the ANS in SCN-driven changes in glucose metabolism is suggested by several studies. First it was shown that electrical stimulation of the SCN resulted in hyperglycemia, which was prevented by blocking α- and β-adrenergic receptors (Fujii et al., 1989; Nagai & Nakagawa, 1992). Moreover, hepatic sympathetic denervation studies revealed that an intact sympathetic innervation of the liver is needed for the SCN to generate a daily rhythm in plasma glucose concentrations (Cailotto et al., 2008; la Fleur et al., 2001) suggesting that rhythmicity of hepatic glucose output is a major determinant of circadian glucose rhythm. The SCN does not directly innervate autonomic motor neurons, but transmits its signal to other areas within the hypothalamus. For the SCN to transmit its rhythms, the PVN is the most important area. For example, BIC or NMDA (agonist of glutamatergic receptors) administered to the PVN, resulted in a prolonged and significant increase in plasma glucose concentrations. Both drugs also increased plasma glucagon concentrations (which may stimulate hepatic glucose output) but did not affect plasma insulin concentrations significantly. Blockade of GABAergic receptors resulted in increased plasma concentrations of corticosterone, which is -like glucagon- known to increase glucose production, whereas stimulating the glutamate receptors did not. These data indicate that it is unlikely that the hyperglycemia induced by the stimulation of PVN neurons is a result of changes in either insulin or corticosterone release, but increased glucagon release might be an attributing factor. Later experiments showed that selective denervation of the sympathetic, but not parasympathetic, autonomic input to the liver completely prevents the hyperglycemic effects of both BIC and NMDA (Kalsbeek et al., 2006). The hyperglycemic effects disappeared in the sympathetically denervated animals notwithstanding pronounced increases of plasma concentrations of glucagon and
corticosterone. Together, these functional studies demonstrate that stimulating neuronal activity in the PVN results in hyperglycemia through activating sympathetic input to the liver. Repeating the above experiments at different times of the day and in SCN-lesioned animals confirmed the SCN as the major site of origin for the GABA and glutamatergic inputs to the PVN (Kalsbeek et al., 2008).

In addition to its (above described) effects on plasma glucose concentrations, the hypothalamus also affects glucose production via the autonomic innervation of the liver. Local administration of BIC in the PVN or the DMH, both areas receiving SCN input, increases hepatic glucose production. The most effective area for increasing hepatic glucose production, however, was the perifornical area (Yi et al., 2009), another area receiving input from the SCN (Vrang et al., 1997) and harboring a dense population of orexin-containing neurons. Thus, for generating the 24h rhythm in plasma glucose concentrations, the SCN transmits its signal to a number of hypothalamic areas important for the regulation of glucose metabolism.

In summary, glucose homeostasis is maintained via balanced changes in glucose uptake and glucose output, controlled by the different nuclei of the hypothalamus. Upon (acute) changes in circulating glucose concentrations, glucose sensing neurons and neuropeptides located in the different areas of the hypothalamus, act to restore glucose homeostasis either directly through autonomic innervation of the liver or indirectly, via modulation of the secretion of glucoregulatory hormones from the pancreas and adrenal glands. The daily fluctuations in basal glucose concentrations are generated by the biological clock in the SCN and its autonomic projections.

Regulation of glucose metabolism beyond the hypothalamus

With regard to the central regulation of glucose metabolism, major focus has been directed to the hypothalamus. Obviously, the hypothalamus receives direct and indirect input from other brain areas. For example, it has been clearly demonstrated that the circuitry between the striatum and hypothalamus is able to influence the hypothalamic control of feeding behaviour (Fulton, 2010; Swanson, 2000). Until now research on this neural network has especially been performed with respect to overconsumption leading to obesity. However, there are studies that support the involvement of these brain areas in the control of glucose metabolism independent of food intake. In this second part of the introduction these areas will be reviewed by considering experimental evidence on i) neural projections, ii) presence of glucosensing neurons and insulin receptors, iii) physiological functionality, and iv) therapeutic interventions in animal models and human subjects.
The ventral tegmental area (VTA)
The ventral tegmental area (VTA) gives rise to the mesolimbic dopaminergic pathway, which consists of dopamine neurons that project to the striatum, amygdala and prefrontal cortex (Vucetic & Reyes, 2010). The VTA contains metabolic sensing neurons (Levin & Routh, 1996) but whether the VTA also contains glucose sensing neurons and whether these are physiologically relevant remains to be elucidated. Interestingly, dopaminergic neurons of the VTA express insulin receptors (Figlewicz et al., 2003). Upon binding to its receptor, insulin is able to increase the expression of the dopamine transporter (DAT), i.e., increasing dopamine reuptake and subsequently decreasing dopamine concentrations and palatable food ingestion (Mebel et al., 2012). In addition, it has been shown that this effect of insulin is diminished when obesity is induced by a high fat diet, suggesting that also at the level of the VTA insulin resistance may occur (Speed et al., 2011). Although the presence of insulin receptors on dopamine neurons has been reported, it is unknown at present whether these dopaminergic neurons are involved in the control of glucose metabolism as well. In addition, no experimental data is available on the effects of therapeutic interventions on glucose metabolism when targeting the VTA.

Considering the ability of insulin to influence mesolimbic dopaminergic tone, subsequent alterations of activity in projection areas of the VTA may direct or indirect, via hypothalamic projections, influence glucose metabolism. These possible pathways will be further discussed below.

Striatum
The nucleus that receives the major part of dopamine projections and forms the relay with the hypothalamus is the nucleus accumbens (NAc). It is part of the ventral striatum and implicated in processing reward related behaviour. Viral tracing experiments revealed a neural connection between the NAc and the pancreas via the parasympathetic nervous system (Buijs et al., 2001). The shell region of the NAc (sNAc) projects to the LHA both via a direct projection and an indirect pathway passing through the ventral pallidum (Zahm & Brog, 1992). The LHA, in turn, projects to the dorsal motor nucleus of the vagus, the ventral lateral medulla and preganglionic spinal cord neurons, all of which project to the pancreas to regulate endocrine pancreatic functions (Buijs et al., 2001; Wu et al., 2004). Besides the pancreas, the LHA-brainstem projections connect to the liver (Berthoud, 2004). It is already well documented that the projection between the sNAc and the LHA plays a role in food directed behaviour (Kelley & Swanson, 1997; Stratford & Kelley, 1999; van der Plasse et al., 2012). But the sNAc, especially considering its projections to the LHA and the pancreas, might also be involved in glucose metabolism.

Interestingly, Papp et al. (2007) showed that the NAc contains glucose monitoring neurons that display a topographical distribution, which points towards a feedback
mechanism of glucose control, as has been described for the VMH (Chen et al., 2010). Recording extracellular single neuron activity during micro-electrophoretic administration of D-glucose, revealed that GI neurons were especially located in the shell, whereas GE neurons were observed in the core region of the NAc. In addition, the authors report on unpublished data about the existence of GE and GI neurons (respectively display firing rates that increase or decrease their response to elevation in blood glucose concentrations) in the primate NAc. Considering that these data parallel those of VMH neurons, which in turn are also involved in regulating glucose metabolism, these NAc areas might be involved in glucose control as well. However, activity of these glucose-sensitive neurons was neither measured during systemic glucose changes nor tested on eliciting a counter-regulatory response. Therefore, the role of these neurons in physiological changes in blood glucose concentrations and/or concentrations of glucoregulatory hormones remains to be elucidated.

Interestingly, changes in plasma insulin concentrations following pharmacological interventions in the NAc have recently been reported. Microinjection of interleukin-1β (IL-1β) into the NAc of rats increased plasma insulin, but not leptin 15 min after injection (Takacs et al., 2012). From these studies, however, it was unclear how these changes occurred and whether there were direct effects on the pancreas or whether this was due to indirect effects of IL-1β on other parts of the brain.

The involvement of the striatum in the insulin response to a glucose challenge can be investigated by dopamine depletion studies. In young male rats, striatal dopamine depletion by infusion of 6-OHDA in the medial forebrain bundle, impaired striatal insulin signalling, but did not alter peripheral concentrations of glucose and insulin (Morris et al., 2008). Interestingly in middle aged rats (16 months old), striatal dopamine depletion, also impaired insulin signalling in the striatum, (showing increased insulin receptor substrate 2 (IRS2), a tendency towards reduced IRS and reduced pAKT protein levels), which was associated with an increased insulin response during a peripheral glucose tolerance test compared to controls. As peripheral glucose concentrations were not changed, this indicates insulin resistance. Whether the effects on plasma insulin following the glucose load were directly due to changes in central dopamine or through other mechanisms was not studied.

Next to these data from animal studies, a link between altered glucose metabolism in the ventral striatum and periphery has been observed in humans. In insulin resistant individuals, an increase in systemic plasma insulin concentration resulted in less glucose uptake, measured by $[^{18}\text{F}]$fluorodeoxyglucose (FDG)-positron emission tomography, in the ventral striatum as compared to insulin sensitive controls. This indicated insulin resistance in a brain area of the corticolimbic system concomitant with peripheral insulin resistance (Anthony et al., 2006).
In summary, animal studies have shown that, with regard to the neural projections of the striatum, in particular the sNAc, may well be involved in the control of peripheral glucose metabolism. This is supported by the detection of glucose sensing neurons in the NAc. However, how these glucose sensing neurons play a role in glucose metabolism remains to be determined. Furthermore, dopamine depletion of the striatum, either with or without a peripheral glucose challenge, decreased striatal insulin signalling and peripheral insulin sensitivity. Also in humans, striatal insulin resistance has been observed in insulin resistant individuals. However, it is difficult to identify whether the changes in normal striatal insulin signalling in insulin resistant states are a cause or consequence of the peripheral insulin resistance and it remains to be elucidated what the central and peripheral pathways are by which the striatum is involved in glucose metabolism. Of interest, dopamine agonists have been shown to have insulin sensitizing and dopamine antagonists insulin resistant effects but whether this is through peripheral or central mechanisms (or both) is unclear. Whether modulating striatal insulin signalling and/or glucose sensing results in clinically significant changes in glucose metabolism remains to be studied.

**Amygdala**

The amygdala consists of distinct but densely interconnected nuclei of which the centromedial nuclei project, amongst other brain regions, to the hypothalamus. Viral tracing experiments revealed that the central amygdala projects to liver and pancreas via the autonomic nervous system (Buijs et al., 2001; Kalsbeek et al., 2006).

The amygdala contains neurons that alter their activity following systemic glucose changes. It has been shown that the amygdala increases activity of the neuronal marker c-Fos in response to subcutaneous administration of 2-DG, indicating the presence of glucose sensing neurons (Dodd et al., 2010). However, in this study c-Fos expression was investigated following 2-DG accompanied by ad libitum access to food, therefore it is difficult to distinguish whether the c-Fos expression is due to glucoprivation induced by 2-DG or due to food intake and subsequent physiological consequences. Interestingly, Zhou et al. (2010) showed that the medial amygdala contains GE and GI neurons of which 54% and 42% respectively express glucokinase, a known critical regulator of glucosensing in pancreatic β-cells as well as in glucose sensing VMH neurons. Specifically, the medial amygdala has been shown to be functional in glucose sensing as local lesioning combined with hyperinsulinaemic hypoglycaemia suppresses the counter-regulatory hormonal response to hypoglycaemia. In addition, the combination of local amygdalar glucoprivation during a moderate hypoglycaemic stimulus amplified the counter-regulatory response, but local glucoprivation in the medial amygdala alone did not. Moreover, this study showed that the medial amygdala innervates the VMH through Urocortin 3 (UCN3) neuronal connections. Approximately 1/3 of these neurons are activated during hypoglycaemia (Zhou et al., 2010). Urocortin
3 is a member of the corticotrophin releasing factor (CRF) family and exerts high affinity for the CRFR2 receptor. Injection of UCN3 in the VMH increased plasma glucose concentrations, and POMC mRNA content in the ARC. Co-localisation of CRFR2 and VGLUT2 (a marker for glutamatergic cells) revealed that the CRFR2-receptor locates on VMH glutamatergic neurons. As the latter project to POMC neurons in the ARC this suggests that UCN3 in the VMH excites POMC neurons in the ARC. Besides the amygdala, the VMH receives UCN3 projection from the PVN and bed nucleus of the stria terminalis (Chen et al., 2011). The role of the UCN3 projections from the amygdala to the VMH in glucose metabolism requires further investigation.

Next to expressing physiological relevant glucose sensing neurons, available research suggests a role for the amygdala in maintaining systemic insulin concentrations. As chemical sensory information from the tongue can be relayed, via the NTS, to the amygdala, the latter is suggested to be involved in the cephalic phase response of insulin (Buijs et al., 2001). This is, however, in contrast to the study of (King et al., 1996) in which female rats were subjected to bilateral electrical lesions of the posterodorsal amygdala. Concentrations of blood glucose and plasma concentrations of corticosterone, ACTH and insulin were assessed during 5 days food restriction and 15 days *ad libitum* feeding following (sham) lesions. No alterations in blood glucose concentrations or concentrations of plasma corticosterone or ACTH were observed in lesioned animals compared to shams neither after food restriction nor after *ad libitum* fed conditions. However, plasma insulin concentrations were elevated in lesioned compared to sham rats after food restriction, which was consistent after 15 days of *ad libitum* feeding (measured 4h after the last meal). After *ad libitum* feeding the lesioned animals significantly gained body weight. Thus, as plasma insulin concentrations were already elevated in lesioned animals during food restriction, this study showed a direct effect of amygdaloid lesion on plasma insulin concentrations, independent of body weight gain.

In addition, changing the insulin signalling cascade within the intact central amygdala has effects on glucose metabolism. When in the central amygdala the serine kinase, PKCθ, that prevents activation of insulin receptor substrate 1 (IRS1) and promotes internalization and degradation of the insulin receptor, is overexpressed unilaterally (reducing insulin action) the hypoglycemic response following intraperitoneal insulin administration is enhanced when rats consume a low fat diet (Park-York et al., 2013). Furthermore, bilateral overexpression of PKCθ in the central amygdala improved glucose tolerance after both a low fat or high fat diet, whereas hepatic triglyceride concentrations were increased together with a small increase in PEPCK gene expression and a decrease in pAMPK gene expression. As the effects of over-expression of PKC in the central amygdala rendered opposing effects on glucose tolerance compared to PKCθ overexpression in the hypothalamus (Benoit et al., 2009),
these data suggests that PKCθ may elicit opposing effects in the central amygdala and the hypothalamus.

Taken together, while the amygdala is proposed to be involved in the cephalic phase response of insulin, this is contradicted by lesion studies, which show that lesioning the amygdala increases plasma insulin concentrations. In addition, local lesion studies combined with central and peripheral hypoglycaemia revealed a role for the amygdala in the counter-regulatory hormonal response. A role for the amygdala in glucose metabolism is further supported by the presence of glucose sensing neurons and UCN3 projections to the VMH, via which the amygdala might exert its effects on glucose metabolism and by studies showing that interference with normal insulin signalling within the amygdala affects glucose tolerance.

**Overall conclusion**

Besides the hypothalamus, corticolimbic brain areas are likely to be involved in the control of glucose metabolism and the counter-regulatory response to hypoglycemia. Although research supporting a functional role is limited, the striatum and amygdala both receive dopaminergic input from the VTA and the mesolimbic dopaminergic tone can be altered in response to insulin. Direct and indirect projections from the sNAc and amygdala to the LHA and VMH respectively (Figure 1) might be involved in regulation of glucose homeostasis. Furthermore, glucose sensing neurons are localized in these corticolimbic areas, which also show reduced insulin signalling concomitant with peripheral insulin resistance. Although more research is needed to elucidate the functional physiological role in glucose metabolism and the mechanism by which corticolimbic brain areas can control glucose metabolism, together these data support a role for the corticolimbic brain areas in the regulation of glucose homeostasis.
Figure 1. Schematic overview of neural projections from corticolimbic brain areas to hypothalamic nuclei that might be involved in the control of glucose metabolism. Both the amygdala and sNAc receive dopaminergic projections from the VTA and express glucose sensing neurons. The sNAc projects direct and indirect (indicated by the dotted line), via the VP, to the LHA, while the VMH receives urocortin 3 projections from the amygdala.