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## **Insulin sensitivity : modulation by the brain**

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GENERAL DISCUSSION  
AND FUTURE PERSPECTIVES





The prevalence of type 2 diabetes mellitus (T2DM) is rising steadily. The world health organization estimates that by the year 2030 more than 5% of the adult population worldwide is suffering from T2DM, as a consequence of the growing obesity epidemic (1). The development of T2DM is the result of both a deficient insulin secretion by pancreatic  $\beta$ -cells and insulin resistance, finally resulting in hyperglycemia. In addition to glucose metabolism, fatty acid (FA) metabolism is disturbed in T2DM. Even though the first evidence that the brain is involved in the control of peripheral glucose homeostasis already dates back from 1855, the role of the brain in the regulation of glucose metabolism has since then been virtually overlooked. Only recently it has been shown that insulin as produced by the pancreas in response to a meal, normalizes plasma glucose levels in part via action in the brain (2;3), suggesting that insulin affects peripheral processes in part via action in the brain. The suprachiasmatic nucleus (SCN) located in the anterior hypothalamus of the brain generates 24 h cycles, so called circadian rhythms, and disturbance of the circadian rhythm, for instance by shift work, has been associated with the development of T2DM (4).

The research described in this thesis was performed to elucidate the role of disturbed circadian rhythm in the development of insulin resistance, to ascertain the role of insulin signaling in the brain on peripheral FA and glucose utilization, and to evaluate an experimental therapy with potential brain action in the treatment of T2DM. The major conclusions and implications of our findings and future perspectives will be discussed here.

## CIRCADIAN RHYTHM DISTURBANCES AND INSULIN RESISTANCE

The rotation of the earth around its axis imparts light-dark cycles of 24 h and by developing an endogenous circadian (*circa*-about and *dies*-day) clock, animals and plants ensured that physiological processes are carried out at the appropriate time of day (5). Many hormones involved in metabolism, such as insulin (6), glucagon (7), adiponectin (8), corticosterone (9), leptin and ghrelin (10) exhibit circadian oscillation. In addition, the circadian clock regulates metabolism and energy homeostasis in liver and other peripheral tissues, by mediating the expression and/or activity of metabolic enzymes and transport systems involved in cholesterol metabolism, amino acid regulation, drug and toxin metabolism, citric acid cycle and glycogen and glucose metabolism (6;11;12). The rhythmic expression and activity of metabolic pathways is mainly the result of coordinated expression of clock genes (*Clock*, *Bmal1*, *Per1*, *Per2*, *Per3*, *Cry1* and *Cry2*) in liver and adipose tissue (13-15). Although each cell in the body harbors its own endogenous clock system, the central circadian clock in mammals that coordinates the peripheral clocks is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus in the brain (16). The SCN clock is composed of multiple, single-cell oscillators, which generate coordinated circadian outputs that regulate rhythms when synchronized (17). Since the SCN oscillation is not exactly 24 h, the circadian pacemaker must be entrained to the external light-dark cycle to prevent drifting out of phase. Light, as perceived by the retina and transmitted to the SCN via the retinohypothalamic tract, is the most potent synchronizer for the SCN (16;18). The SCN transmits its rhythmic signal to

the peripheral oscillators via hormones and the autonomic nervous system (19-21), thereby influencing nearly all aspects of physiology and behavior, including sleep-wake cycles, cardiovascular activity, endocrine system, body temperature, renal activity, physiology of the gastrointestinal tract and hepatic metabolism (16;22). Complete ablation of the SCN abolishes circadian rhythmicity in the periphery as it leads to a loss of synchrony among individual cells and dampening of the rhythm at the population level (23;24). As a result, SCN lesioned (SCNx) rats do not show a circadian rhythm in, for example, glucose plasma levels and glucose uptake (6) or leptin regulation (25).

In **chapter 2** we disrupted circadian rhythmicity by constant light exposure, which resulted in decreased (~50%) SCN output. The relatively mild decrease in SCN rhythm amplitude completely abolished peripheral circadian rhythms in energy metabolism and insulin sensitivity. Determining the oscillatory expression of glycoregulatory genes in liver and muscle could shed more light into how the decrease in SCN output results in complete loss of rhythmicity in peripheral processes. Disruption of circadian rhythm by constant light had an immediate effect on SCN output as well as on energy metabolism. As a result of increased food intake and decreased energy expenditure, constant light exposure stimulated body weight gain instantaneously. This suggests that short term alterations in circadian rhythm, for instance caused by (social) jetlag and disrupted sleep in humans, can have immediate effects on homeostasis, thereby contributing to development of secondary metabolic pathophysiology. Indeed, a single night of partial sleep deprivation acutely reduces energy expenditure in healthy men (26) and induces insulin resistance in type 1 diabetic patients (27), as well as in healthy subjects (28). It should be explored whether interventions aimed at optimizing sleep duration can be beneficial for stabilizing glucose levels in patients with diabetes. As disturbances in circadian rhythms in humans are also associated with dyslipidemia and cardiovascular morbidity and mortality (29-31), and mice mutant for certain clock genes develop hyperlipidemia and increased vascular injury (32-34), it will be of interest to elucidate the involvement of the circadian clock in lipid metabolism and to determine the effect of disturbed circadian rhythm in the development of dyslipidemia and atherosclerosis.

In **chapter 3** we investigated the role of the SCN in more detail, by studying the effect of SCN lesions on energy metabolism and insulin sensitivity. Thermic ablation of the SCN (SCNx) completely disrupted the circadian pattern in energy metabolism, resulting in a higher percentage of food intake during the day. In line with previous studies showing that a shift in energy intake from the nocturnal part of the day to the diurnal part of the day results in adiposity (35-37), the SCNx mice showed a mild increase in body weight, due to increased fat mass. Even though the SCNx mice were only marginally overweight, hepatic insulin sensitivity was severely impaired. The hepatic insulin resistance in these SCNx mice could be the result of disrupted SCN mediated control of glucose production, as the SCN has been shown to directly control glucose production by the liver via innervation (38).

Both **chapter 2** and **3** indicate that disturbances in circadian rhythm can contribute to the development of T2DM. The set-up in the two studies was similar: insulin sensitivity was determined by hyperinsulinemic-euglycemic clamp technique in C57Bl/6J male mice five weeks after the intervention, either constant light exposure or ablation of the SCN, was

started. The chow fed control mice from both studies had a similar body weight and also similar insulin sensitivity. The reduced SCN output, induced by constant light exposure, immediately stimulated weight gain by increasing food intake during the subjective day. Later on, the food intake in constant light was decreased and the weight gain induced by the light intervention stabilized. SCN ablation resulted in complete arrhythmia almost immediately, but this was not reflected in an immediate body weight gain. The body weight development did not differ between SCNx and control mice for the first three weeks after the operation, but when the hyperinsulinemic clamp was performed, SCNx mice were slightly, but significantly heavier. Even though the SCNx mice had a similar body weight compared to mice in constant light on a high-fat diet, the SCNx mice were very insulin resistant, whereas the mice in constant light on a high-fat diet had only lost their circadian variation in insulin sensitivity. Therefore, it will be interesting to determine how much input from the SCN is needed to prevent the development of insulin resistance. As it is now established that disturbances in circadian rhythmicity can contribute to the development of obesity and T2DM, patients with mild rhythm disturbances, such as elderly and shift workers, can benefit from prevention and treatment programs to synchronize behavior and endogenous phase.

Patients treated for large pituitary tumors have disturbed sleep duration and quality, and disturbed circadian movement rhythm (39). These patients also have a higher prevalence of metabolic risk factors, such as dyslipidemia and insulin resistance, as compared to the general population, despite optimal replacement therapy of hypopituitarism (40-43). The sleep disorders as well as the metabolic abnormalities in these patients could be the result of damage to the SCN by the initial tumor, as we show in **chapter 3** that lesions of the SCN completely disrupts circadian rhythm and severely impairs hepatic insulin sensitivity. It needs to be established whether normalization of sleeping patterns in these patients (for example by maintaining a regular schedule for going to bed and waking up and engaging in stimulating activities and light exposure immediately after waking up (44)), can also reverse the metabolic abnormalities.

## THE ROLE OF INSULIN SIGNALING IN THE BRAIN

Insulin is secreted by pancreatic  $\beta$ -cells after a meal to normalize glucose levels by inhibiting endogenous glucose production (EGP) and stimulating glucose disposal by peripheral tissues. In part, the effects of circulating insulin are mediated by the brain, as neuron-specific insulin receptor knock-out (NIRKO) mice develop mild insulin resistance and elevated plasma insulin levels in association with obesity (45). Consistent with these data, decreased hypothalamic expression of the insulin receptor (IR) elicits insulin resistance in rats (2). A large body of work has addressed the effect of central insulin administration on EGP. For example, central infusion of insulin has been shown to suppress endogenous glucose production (3) and antagonism of insulin signaling in the brain impairs the ability of circulating insulin to suppress EGP (3). However, much less is known on the central regulation and the downstream mechanism by which central insulin affects peripheral glucose disposal and how this is regulated in an insulin resistant model.

In **chapter 4**, we show that insulin-stimulated glucose uptake during hyperinsulinemic clamp conditions is dependent on insulin signaling in the brain. Blocking insulin signaling in the brain by intracerebroventricular (i.c.v.) administration of the ATP-sensitive potassium ( $K_{ATP}$ ) channel blocker, tolbutamide, inhibited insulin-stimulated glucose uptake by skeletal muscle, but not heart or adipose tissue. Previously, it was shown that i.c.v. administered insulin increases glucose storage in muscle as glycogen (46). Together with the results obtained in **chapter 4**, a concept emerges of an insulin-dependent central pathway targeted at skeletal muscle.

In the brain,  $K_{ATP}$  channels with different properties are found in various cell types: glial cells (47) and dorsal vagal (48), hippocampal (49), and hypothalamic neurons, including proopiomelanocortin (POMC)- and agouti-related peptide (AgRP)/neuropeptide Y (NPY)-expressing neurons of the ARC (50;51). In previous studies, it has been shown that  $K_{ATP}$  channels are activated downstream of the insulin receptor. I.c.v. administration of  $K_{ATP}$  channel blockers abolish the central effects of insulin on EGP and prevent in part the suppression of EGP by circulating insulin (3). Moreover, activation of  $K_{ATP}$  channels in the ARC is sufficient to lower blood glucose by inhibition of hepatic glucose production and gluconeogenesis (52;53).  $K_{ATP}$  channels are heterooctameric proteins composed of inwardly rectifying  $K^+$  channel subunits (KIR6.1 or KIR6.2) and regulatory sulfonylurea receptor (SUR) subunits (54;55). Interestingly, mice lacking the SUR1 subunit of the SUR1/Kir6.2  $K_{ATP}$  channel show impaired suppression of hepatic gluconeogenesis and EGP by insulin (52). It remains to be determined how the insulin-signaling cascade leads to the activation of  $K_{ATP}$  channels. A possible mechanism could be via the regulation of the intracellular ATP/ADP ratio, either by increasing levels of glucose or AMP-activated kinase (AMPK). A reduction of the ATP/ADP ratio results in closure of  $K_{ATP}$  channels, consecutive depolarization, and increased neuronal firing, thereby exerting their effects on second-order neurons.

The orexigenic AgRP/NPY neurons and the anorexigenic POMC-neurons both express insulin receptors and are targeted by insulin (45;56). Interestingly, i.c.v. NPY or AgRP do not stimulate muscle glucose uptake from blood (57), whereas acute and chronic i.c.v. infusion of a POMC agonist enhances insulin-stimulated muscle glucose uptake (58-60). Therefore, insulin may signal through POMC, and not AgRP/NPY, to regulate muscle glucose uptake. As glucose utilization in skeletal muscle can be stimulated by sympathetic activity and i.c.v. insulin in rats increases sympathetic activity to the hind limb (24), it will be of interest to determine whether insulin action in POMC neurons regulates sympathetic activity, thereby stimulating glucose uptake by muscle. It will also be of interest to determine whether activation of  $K_{ATP}$  channels is sufficient to stimulate glucose uptake by muscle.

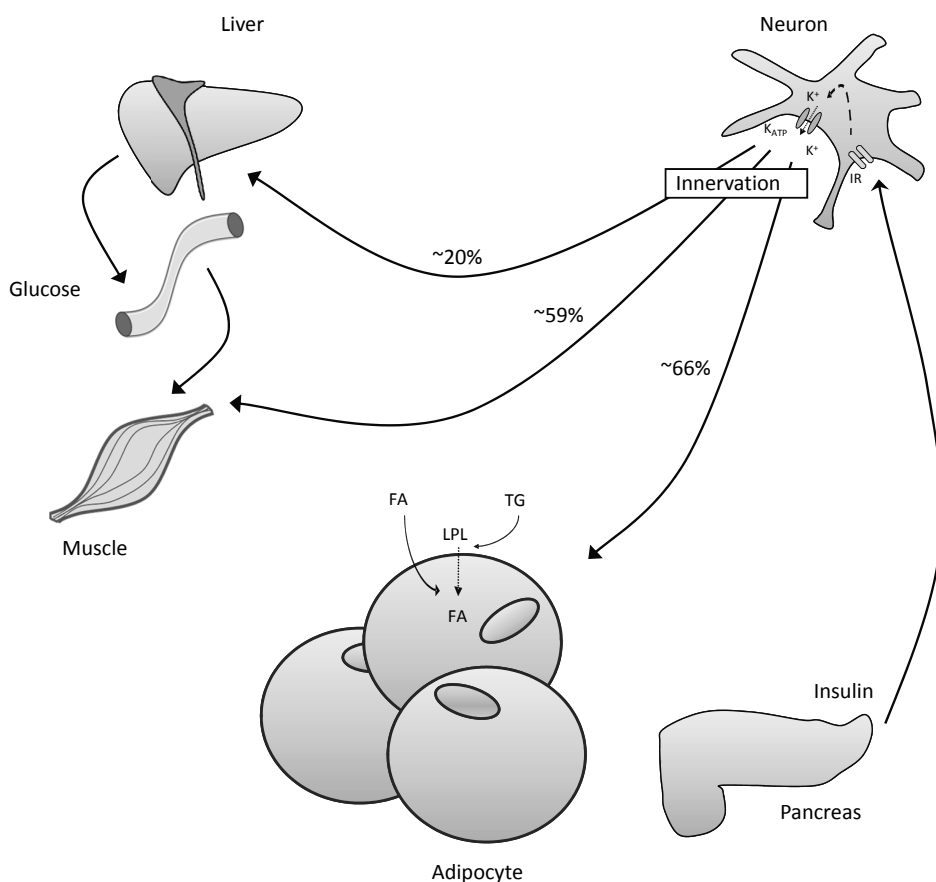
Upon neural stimulation, skeletal muscles are stimulated to take up glucose. This uptake of glucose most probably involves the glucose transporter 4 (GLUT4). GLUT4 is a 12-transmembrane protein expressed in muscle and adipose tissues that catalyzes the transport of glucose across the plasma membrane via an ATP-independent, facilitative diffusion mechanism. Upon stimulation, GLUT4 is recruited to the plasma membrane, resulting in 2-10-fold increase in the surface level of GLUT4. The translocation of GLUT4 to the plasma membrane is responsive to hormones such as insulin and by energy-demanding conditions such as exercise and hypoxia. The insulin-stimulated glucose uptake by muscle involves the phosphatidylinositol 3-kinase (PI3K) pathway,

whereas glucose uptake in response to exercise and hypoxia is PI3K-independent, but involves AMPK activation. In **chapter 4**, we show that under hyperinsulinemic conditions glucose uptake by skeletal muscle is stimulated, which coincides with an increase in insulin signaling in muscle. Blocking insulin signaling in the brain by i.c.v. administration of the  $K_{ATP}$  channel blocker tolbutamide inhibited insulin-stimulated glucose uptake by muscle. This inhibition of insulin-stimulated glucose uptake occurred independent of a change in insulin signaling in muscle, but also independent of a change in AMPK activation. So, the brain-mediated glucose uptake by muscle does not involve the PI3K pathway or the “alternative pathway” (involving AMPK). Therefore, more research is needed to determine how insulin signaling in the brain can affect insulin-stimulated glucose uptake by muscle.

Insulin not only regulates glucose metabolism, but also stimulates fat storage in adipose tissue. In **chapter 6**, we show that insulin-stimulated uptake of FA by white adipose tissue (WAT) is also in part dependent on central insulin signaling. I.c.v. administration of the  $K_{ATP}$  channel blocker tolbutamide abolished the central effects of insulin on FA uptake by WAT and prevented, in part, the circulating insulin-stimulated FA uptake by WAT. By employing the dual tracer methods described previously (61), we made a distinction between FA derived from plasma triglyceride (TG) and FA derived from plasma albumin. Both peripheral and i.c.v. administered insulin stimulated the retention of both sources of plasma FA in WAT. This insulin-stimulated FA uptake by WAT was accompanied by a decreased half-life of TG, reflecting increased turnover of plasma TG. Interestingly, these acute effects of i.c.v. insulin administration on plasma TG and FA fluxes towards WAT, together with recent data showing that brain insulin suppresses intracellular lipolysis and stimulates lipogenesis in adipocytes (62), provide an explanation for the finding that chronic i.c.v. insulin administration increases fat mass (63). The indirect effect of insulin on fat storage via the brain might involve alterations in the activity of the autonomic nerves projecting towards WAT. Parasympathetic denervation of WAT reduced FA uptake in WAT by 36% during hyperinsulinemic clamp conditions in rats (64), suggesting that an increase in parasympathetic activity towards WAT might be involved in insulin-stimulated fat storage. In **chapter 6** we show that in CD36-deficient mice, i.c.v. insulin was unable to stimulate fat storage, suggesting a role for the long-chain FA transporter CD36 in i.c.v. insulin stimulated FA uptake. Accordingly, AMPK, which can stimulate FA uptake by promoting translocation of CD36 from intracellular pool to the plasma membrane (65), was activated in WAT upon i.c.v. insulin administration. A concept emerges of an insulin-dependent central pathway, leading to parasympathetic innervation of WAT and via CD36 translocation following AMPK activation resulting in FA uptake by WAT. Insulin also affects FA metabolism by acutely decreasing VLDL secretion by the liver (66;67). Therefore it will be interesting to determine whether this effect is also mediated by insulin action in the brain.

Combining data obtained in **chapter 4** and **chapter 6** indicates that circulating insulin affects glucose and FA metabolism in part via action in the brain. These effects via the brain are organ specific: central insulin signaling is involved in glucose uptake by skeletal muscle, but not heart or WAT, and central insulin signaling is involved in FA uptake by WAT, but not heart, muscle, liver or brown adipose tissue (BAT). The contrasting effects of central insulin suggest a branched-pathway model of hypothalamic insulin signaling in which insulin (in POMC

neurons, via sympathetic activity?) stimulates glucose uptake by muscle, whereas insulin (via parasympathetic activity?) promotes FA uptake in WAT. A tentative model of central insulin signaling is depicted in Fig. 1.



**Fig. 1.** Tentative model of central insulin signaling. See text for explanation.

The observations in **chapter 4** and **chapter 6** stress the role of central insulin signaling in normal physiological conditions in maintaining glucose and lipid homeostasis. However, high-fat feeding abolished the inhibitory effect of i.c.v. tolbutamide, suggesting central insulin resistance. Our *in vivo* observations in diet-induced obese mice extend *in vitro* observations, showing that insulin activates  $K_{ATP}$  channels in glucose responsive neurons of lean, but not of obese rats, suggesting that  $K_{ATP}$  channels are already inhibited in the insulin resistant state (68-71). The ability of the brain to sense peripheral inputs and maintain metabolic homeostasis is impaired upon increased caloric intake, thereby contributing to the pathophysiology of obesity

and T2DM. New therapeutic interventions restoring brain insulin action in the hypothalamus of obese individuals could improve peripheral insulin sensitivity and plasma glucose levels.

It is now shown that circulating insulin affects peripheral processes, such as glucose and FA metabolism, in part via action in the brain. The fact that part of insulin's effects are controlled by the brain could reflect a similar situation for other hormones, for instance thyroid hormones (TH). Thyroid hormones (TH) are crucial regulators of metabolism. In **chapter 7** we show that TH status determines energy expenditure and tissue-specific changes in TG-derived FA uptake. TH status also affects glucose homeostasis: endogenous glucose production is increased upon high TH levels and reduced upon decreased TH levels (72-75). Recently it has been shown that TH administration directly in the brain mimics the effect of peripheral TH on glucose metabolism (76;77) and that both peripheral as well as central effects of TH on glucose production depend on sympathetic projections to the liver (77;78). This suggests that TH may affect glucose production in part via action in the brain. At this stage, however, it is unknown how these results reflect the human situation and whether hypothalamic bioavailability of TH is altered in diabetic patients.

## PHARMACOLOGICAL INTERVENTION IN THE BRAIN

The question arises whether the loss of insulin's actions in the brain induced by high-fat feeding are amendable to pharmacological intervention. In **chapter 5** we studied the effects of topiramate on insulin sensitivity as assessed by hyperinsulinemic-euglycemic clamp analysis. Topiramate, a sulfamate-substituted derivative of the monosaccharide D-fructose (79), is a broad-spectrum antiepileptic drug with potentially additional neurotherapeutic applications such as bipolar disorder and migraine (80;81). Its precise mechanism of action is unknown, although the antiepileptic effects of topiramate are mediated through at least six mechanisms of action within the central nervous system: (i) enhancement of GABA-ergic activity (82;83), (ii) inhibition of kainite/AMPA receptors (84), (iii) inhibition of voltage-dependent sodium channels (85), (iv) inhibition of high-voltage-activated calcium channels (86), (v) increase in potassium conductance (87) and (vi) inhibition of carbonic anhydrase (88). Besides its antiepileptic action, topiramate usage is associated with decreases in body weight. The reduction in body weight by topiramate treatment has been shown in epileptic patients as well as in obese, non-epileptic, persons (89-91). Although loss of appetite resulting in reduced caloric intake can account for initial reductions in body weight, other mechanisms might be involved in the long-term effects of topiramate on weight, as weight loss continued after caloric intake returned to baseline levels (91). Even though the half-life of topiramate is lower in rodents than in humans (~1-2 h), several studies have confirmed that topiramate also reduces food intake and body weight in lean as well as in obese mouse and rat models (92;93). Studies in obese, diabetic rats demonstrate that topiramate treatment reduces plasma glucose levels and improves insulin sensitivity independent of weight loss (94;95). In line with this, we document improved insulin sensitivity in our mice in absence of a reduction in body weight. This improvement in insulin sensitivity was present in (cardiac and skeletal) muscle and adipose tissue, but not in liver. As the concentration of topiramate in plasma correlates with that in cerebral spinal fluid (CSF) (96) and topiramate's

effects on body weight, body composition and energy metabolism are associated with altered neuropeptide expression in the hypothalamus (97), whereas topiramate does not have a direct effect on insulin sensitivity in muscle cells, we hypothesized that the brain mediates the insulin sensitizing effects of topiramate. Since tolbutamide inhibits activation of neuronal  $K_{ATP}$  channels by insulin in insulin sensitive subjects, tolbutamide was used to demonstrate the involvement of the brain in the insulin sensitizing effect of topiramate. Central administration of tolbutamide in our mice had no effect on insulin sensitivity during hyperinsulinemic-euglycemic clamp, suggesting insulin resistance in the brain induced by high-fat feeding. I.c.v. tolbutamide in topiramate treated mice inhibited the insulin sensitizing effect of topiramate, suggesting that the improved insulin sensitivity by topiramate originates from an insulin sensitizing effect in the brain directed at muscle and adipose tissue but not liver. This improved insulin sensitivity in muscle and adipose tissue, but not in liver, has previously been associated with enhanced AMPK phosphorylation in muscle but not in liver (92). As  $\alpha$ -adrenergic stimulation enhances AMPK phosphorylation, the increased glucose uptake by peripheral organs might be related to increased sympathetic nervous system (SNS) activity (98).

Topiramate treatment has some negative side-effects that need to be overcome before it can be widely used in the clinics as an antidiabetic drug. Drowsiness, dizziness, fatigue and nervousness are common side-effects. Furthermore, determination of body composition by dual-energy X-ray absorptiometry in our mice showed a significant reduction in bone mineral density, in line with reports that topiramate treatment in epileptic children and young women results in hypocalcaemia, increased bone turnover and reduced bone mineral density (99;100), suggesting that topiramate has negative long-term effects on bone development. Taken together, topiramate improves insulin resistance by restoring insulin sensitivity in the brain and this feature renders topiramate as an exciting new drug when negative side effects are overcome, to treat diabetes by itself or in combination with classic antidiabetic drugs.

Other drugs that improve insulin sensitivity probably in part via action in the brain are GLP-1 analogues. GLP-1 is a gut hormone, secreted in response to food. GLP-1 has many beneficial actions: it enhances glucose-stimulated insulin secretion, improves blood glucose profiles in type 2 diabetes patients, reduces body weight and food intake and slows gastric emptying. However, the half-life of native GLP-1 is only a few minutes in humans. Therefore, pharmaceutical companies are developing GLP-1 analogues that have the same beneficial qualities, but with a longer half-life. GLP-1 improves insulin sensitivity in part via activation of GLP-1 receptors in the brain (101;102). The neuronal circuits that are activated by peripheral administered GLP-1 (or its analogues) remain to be identified. One mode of action of GLP-1 could be a modulation of the NPY pathway in the hypothalamus, as i.c.v. GLP-1 completely prevents the orexigenic effects of NPY (103). Furthermore, GLP-1 has been reported to reduce NPY neuronal activity (104). Since NPY has been linked to insulin resistance, chronic administration of GLP-1 or any of its analogues may antagonize NPY-induced insulin resistance. GLP-1 can also improve insulin sensitivity by acting in the brainstem. Located in the brainstem, the nucleus of the solitary tract (NTS) receives vagal afferent input from the gastrointestinal tract. GLP-1 (or its analogues) can activate neurons in the NTS via afferent vagal input (105;106). Elucidating the precise molecular events in the brain that underlie the effects of GLP-1 (and its analogues) will lead to new or improved therapeutic agents.

## CONCLUDING REMARKS

As the brain is clearly involved in maintaining peripheral homeostasis, experimental therapies with action in the brain should be considered as promising strategies for the treatment of T2DM and T2DM-associated complications. Disruptions in circadian rhythm, resulting in altered output by the SCN, have an immediate effect on energy metabolism and insulin sensitivity. It has become clear that the brain is able to directly sense circulating hormones and nutrients that provide the brain with information regarding the metabolic status of the body. Thyroid hormones affect glucose metabolism, and possible other processes as well, possibly via action in the brain. Insulin's action in glucose and FA metabolism results in part via central mechanisms. High-fat diet induces not only peripheral insulin resistance, but also central insulin resistance, which can contribute to the pathophysiology of T2DM. Restoring insulin sensitivity in the brain, as we have observed with topiramate, may be a promising new target for T2DM therapies or can extend the action of current therapies.

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