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Review

Brain insulin, energy and glucose homeostasis; genes, environment and metabolic pathologies

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Abstract

The central nervous system is essential in maintaining energy and glucose homeostasis. In both animals and humans, efficient cerebral insulin signalling is a pivotal control element in these pathophysiological processes. The action of insulin in the brain is under a multilevel control *via* metabolic, endocrine and neural signals induced by nutrients, integrated mainly by the hypothalamus. Of particular interest is the interaction of insulin with the anabolic and catabolic neuroregulators. The anorexic peptides insulin, leptin and the neurotransmitter serotonin share common signalling pathways involved in food intake, in particular the insulin receptor substrate, phosphatidylinositol-3-kinase (PI3K) pathway. The dialogue of neurotransmitters and peptides *via* this signalling pathway is potentially of major importance in the pathophysiology of the brain in general and specifically in the regulation of feeding behaviour. At this time, a new concept in the aetiopathology of type 2 diabetes is immerging. This concept proposes that the combination of defective pancreatic beta-cell function and insulin resistance not only in classical insulin target tissues but in every tissue, contributes to the onset of the disease. It highlights the importance of the disruption of cerebral insulin signal transmission and its direct relation to metabolic diseases. Impaired brain insulin signalling, a link coupling obesity to diabetes, may be related to either genetic factors, or environmental factors such as stress, over or under-feeding and unbalanced diets: such factors may work either independently or in concert. Current approaches used for the prevention and treatment of type 2 diabetes are not adequately effective. Most of the anti-diabetic therapies induce many adverse effects, in particular obesity, and thus may initiate a vicious cycle of problems. In order to develop new, more efficient, preventive and therapeutic strategies for metabolic pathologies, there is an urgent need for increased understanding of the complexity of insulin signalling in the brain and on the interactive, central and peripheral effects of insulin.

Keywords: Insulin; Leptin; Serotonin; Nutrients; Obesity; Diabetes

Contents

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1. Introduction

The understanding that mechanisms regulating vital physiological processes such as feeding acquisition, energy and glucose homeostasis, are largely operating in the central nervous system, has added a new dimension in the investigation of the physiopathology of metabolism. An intimate coupling of neuronal mechanisms regulating body weight and glucose metabolism is revealed (Prodi and Obici, 2006; Schwartz and Porte, 2005). While it was long considered that the brain was insulin-independent, it has now been established that brain insulin plays a crucial role in the regulation of metabolism, and that altered insulin action in the brain is directly involved in metabolic diseases such as obesity, diabetes or the metabolic syndrome. Recent concepts regarding the development of type 2 diabetes while including the role of malfunctions at the level of beta cell, muscle and liver, also take into account the involvement of other tissues and the importance of the brain.

Both a protein identical to that produced by the pancreatic beta cells and specific insulin receptors are widely distributed in the networks of the central nervous system related to feeding, reproduction or cognition (Baskin et al., 1983; Bruning et al., 2000; Gerozissis, 2003; Havrankova et al., 1979; Schechter et al., 2005; 1996; Schulingkamp et al., 2000; Schwartz et al., 1992). Insulin receptors are present in particularly high concentrations in neurons, and in lower levels in glia. The messenger RNA of insulin receptors is abundantly localized in neuronal somata, and receptor protein is found in both cell bodies and synapses (Schwartz et al., 1992; Zhao and Alkon, 2001). The major molecular structure and most of the properties of brain insulin receptors are identical to peripheral insulin receptors (Wozniak et al., 1993).

One controversial topic in brain insulin research remains that concerning its origin: whether it derives from the periphery, from local sources or from both (Banks, 2004; Devaskar et al., 1994; Gerozissis, 2003, 2004; Schechter et al., 1996, 2005; Steen et al., 2005; Woods et al., 2003). The traditional view, largely reflected in the literature, is in favour of the concept that brain insulin is exclusively of pancreatic origin (Banks, 2004; Schwartz et al., 1992). Transport of blood insulin in the brain has been convincingly demonstrated. Insulin can enter into circumventricular regions which lack a blood brain barrier, and can cross the blood brain barrier *via* insulin receptor-mediated active transport (Baura et al., 1993; Woods et al., 2003). However, local production of some portion of the insulin present in the brain cannot be excluded. Suggestions of possible insulin biosynthesis in the brain were based at first, on indirect arguments (Baskin et al., 1985; Gerozissis et al., 1993, 1997, 2001; Havrankova et al., 1979; Orosco et al., 1995). Additional recent data (Schechter et al., 2005), and evidences based on cultured neurons and molecular biology approaches are in favour of a *de novo* local insulin production in the brain. Reverse transcription-polymerase chain reaction (RT-PCR) and *in situ* hybridization approaches clearly indicate that insulin and preproinsulin messenger RNA is expressed in the foetal, newborn and the adult rodent brain, and has been detected in the hypothalamus, the cortex and the hippocampus (Clarke et al., 1986; Devaskar et al., 1994; Gerozissis et al., 2007b; Grunblatt et al., 2007; Hrytsenko et al., 2007; Schechter et al., 1990, 1994, 1996; Steen et al., 2005). The proposal that there are two sources of insulin in the brain is consistent with the above experimental data and reconciles numerous paradoxes reported in the literature (Gerozissis, 2003, 2004; Schechter et al., 2005; Zhao and Alkon, 2001; Zhao et al., 2004).

That the brain itself synthesizes some portion of the insulin detected locally is not an unusual occurrence. Other peptides produced peripherally have also been demonstrated to be synthesized in the central nervous system: Glucagon-like peptide-1 involved in the pathogenesis of diabetes, obesity, and stress is produced both in the human and rat intestine and brain (Bojanowska, 2005). Prolactin is not only synthesized in the pituitary gland, but also within the central nervous system, the immune system and the uterus. Moreover, its biological actions are not limited solely to reproduction: it has been shown to control a variety of behaviours and even play a role in homeostasis (Freeman et al., 2000). Ghrelin, a peptide hormone that is involved in the hypothalamic regulation of energy homeostasis, and is produced by the stomach, is also produced by hypothalamic neurons; further, ghrelin receptors are expressed in various regions of the brain (Nakazato et al., 2001). Finally, cholesystokinin which is involved in food intake regulation, is produced in both the intestine and the central nervous system (Beinfeld, 1983). Why should insulin be an exception? Those who favour the position that insulin is not produced in the central nervous system point to the very low basal levels of insulin biosynthesis in the brain. However, peptides are mainly produced in small amounts at baseline conditions. Increased release is induced in response to stimuli (Hökfelt et al., 2002). Brain insulin could follow the same pattern.

In this article we will focus mainly on four major points: 1) the cellular and molecular mechanisms involved in the biological effects of insulin in the brain and its complex interactions with main neuroregulators of energy and glucose metabolism, 2) the impact of genetic predisposition and unbalanced nutrition on the mechanisms of the action of insulin in the brain, 3) the hypothesis that altered cerebral insulin mechanisms contribute to metabolic diseases and 4) some novel perspectives for improvements in the treatment of metabolic diseases, based on knowledge derived from the study of brain insulin.

2. Central control of homeostatic mechanisms by insulin: energy balance and metabolism

Insulin has two important functions that relate to overall metabolic homeostasis: the maintenance of adequate energy stores to allow for development, growth, and reproduction, and the regulation of plasma glucose. The understanding that the central nervous system plays a key role in both of these functions, and the concept that both body weight and plasma glucose are critically regulated by the same hormone is the subject of ongoing research. The importance of insulin in brain functioning has been studied far less than its role in the periphery. Nevertheless, the pleiotropic nature of the action of insulin in the central nervous system has been the subject of numerous reviews (Gerozissis, 2003, 2004; Könner et al., 2007; Morton et al., 2006; Niswender and Schwartz, 2003; Plum et al., 2006; Woods et al., 2000; Wozniak et al., 1993; Zhao et al., 2004).

Despite wide fluctuations in physical activity, and in the composition and amount of food ingested daily, under most circumstances, in adult mammals, energy intake matches energy expenditure over time. The central control of energy balance and the adjustment of both food intake and energy expenditure in response to a wide range of environmental factors, metabolic and hormonal signals occur mainly in the hypothalamus. Insulin, in interaction with the other regulatory peptides and neurotransmitters (Fig. 1) can activate processes related to feeding behaviour, learning and memory, and is potentially involved in the communication within brain structures, in particular the hypothalamus and the limbic system. At present, the concept that an efficient action of insulin in the brain is necessary for the maintenance of energy, glucose and fat homeostasis, is largely accepted (Fehm et al., 2006; Gerozissis, 2003, 2004; Lam et al., 2005; Morton et al., 2006; Obici et al., 2002a,b; Plum et al., 2005; Schwartz and Porte, 2005; Woods et al., 2000).

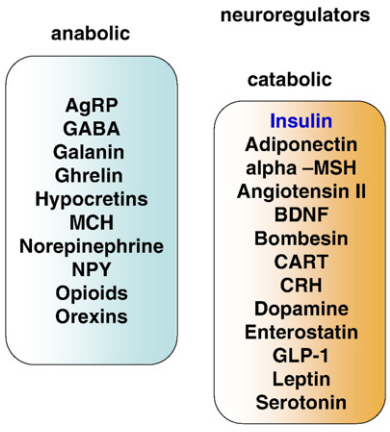

Fig. 1. Positive and negative neuroregulators of energy and glucose homeostasis. AgRP = Aguti related protein, alpha-MSH = alpha-melanocyte-stimulating hormone, BDNF = Brain-derived neurotrophic factor, CART = Cocaine- and amphetamine-regulated transcript, CRH = Corticotropin-releasing hormone, GABA = gamma-aminobutyric acid, GLP-1 = Glucagon-like peptide-1, MCH = Melanin concentrating hormone, NPY = Neuropeptide Y.

Insulin in the brain induces both short and long term effects on feeding behaviour and body weight. The anorexigenic and leptogenic (loss of body weight) action of insulin has been revealed by administering the hormone in the central nervous system of both rodents and primates and by disrupting insulin receptors directly in the brain (Air et al., 2002; McGowan et al., 1990; Nicolaïdis, 1978; Obici et al., 2002b; Schwartz et al., 1992; Strubbe and Mein, 1977; Woods et al., 1979). The survival of an animal depends largely on how efficiently it can regulate its supply and use of metabolic fuels and essential nutrients. Feeding behaviour depends on the ability of the brain to encode and retain in memory a variety of information about experiences with food. Rodents maintained on a single daily meal provided at a fixed time of the day, within a few days exhibit food anticipatory activity during the hours immediately preceding feeding time: changes in the action of orexins and melanocortins, increased locomotor activity (Benoit et al., 2003; Mieda et al., 2004). When neuropeptide Y is administered centrally, meal-anticipatory responses are elicited (Drazen et al., 2005). In expectation of the meal animals synthesize and secrete regulators of food intake and cognition, including hypothalamic insulin, serotonin, ghrelin and neuropeptide Y (Drazen et al., 2006; Gerozissis, 2003, 2004; Gerozissis et al., 2004; Orosco et al., 1995; Yoshihara et al., 1996). We hypothesize that brain insulin, in interaction with serotonin, leptin and melanocortins, is involved in cognitive processes related to anticipation of meal time (Benoit et al., 2003; Gerozissis, 2003, 2004; Gerozissis et al., 2001, 2004).

In recent years, the idea that glucose metabolism throughout the body is coordinated by the brain, has gained growing support (Pocai et al., 2005). The hypothalamus plays a pivotal role in the control of both the energy and glucose balance. Efficient action of insulin in the brain is necessary for glucoregulation. An intact hypothalamic insulin signalling mechanism is required for the full inhibitory effect of systemic insulin on suppressing endogenous glucose production (Obici et al., 2002a; Prodi and Obici, 2006). An alteration within the central nervous system/liver circuit can contribute to diabetic hyperglycemia (Pocai et al., 2005). Additionally, Perrin and collaborators (2004) demonstrated that in the muscle, glycogen synthesis can be increased by the cerebral action of insulin *via* a mechanism potentially involving AMP-activating kinase.

3. Insulin regulation in the brain

3.1. Multifactorial and multilevel control of insulin in the brain

Brain insulin is subject to a multifactorial control which may be exerted at various levels. The biosynthesis and secretion of the hormone in the pancreas, along with its transport into the brain, storage, stability and its potential local production and release in the central nervous system, may be affected by both genetic factors and environmental factors. These factors may have an effect either directly or *via* modification of metabolites, circulating hormones, regulatory peptides and neurotransmitters, and thus influence insulin (Fig. 2). The biological effects of insulin depend on the availability of the hormone in the brain,

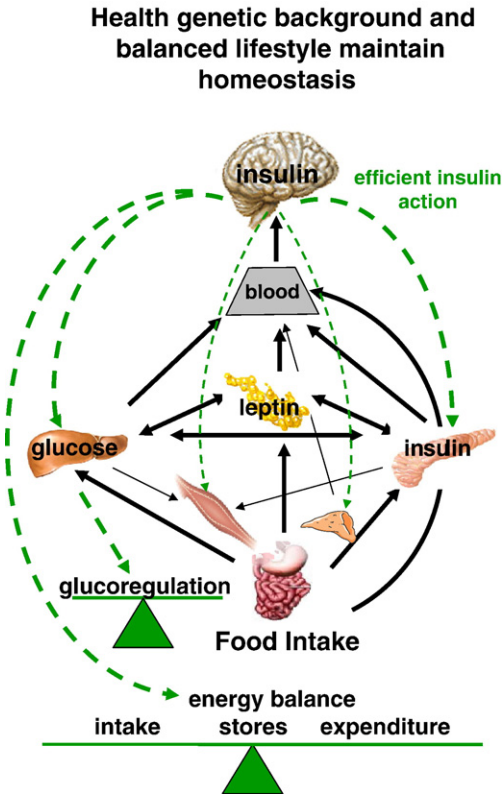

Fig. 2. Nutritional, metabolic, neural and endocrine signals control insulin production and secretion and have a direct impact on circulating insulin levels and on the transport of blood insulin in the brain. Integrated in the brain, they control ingestive and appetite behaviours and energy expenditure *via* a negative regulatory loop. Insulin has a major biological role in controlling those processes. Under physiological conditions, in a health genetic background and balanced life style, hypothalamic insulin and its partners reduce food intake postprandially and contribute in maintaining energy and glucose homeostasis.

its binding to specific receptors and the activation of its intracellular signalling pathways.

Circulating insulin concentrations are proportional to body fat content, but are also greatly influenced by recent energy intake and dietary macronutrient content (Havel, 2001; Rouch et al., 2005; Schwartz and Porte, 2005; Woods et al., 2000). Food intake, fasting, refeeding, and the composition of the diet influence the rate of insulin entry in the brain (Kaiyala et al., 2000; Strubbe et al., 1988; Woods et al., 2003). In animals and humans, eating increases the concentration of insulin in the cerebrospinal fluid and its release in the hypothalamus whereas fasting decreases brain insulin levels (Gerozissis et al., 1998; Orosco et al., 1995; Schwartz et al., 1992). The nature of macronutrients modifies specifically the extracellular hypothalamic insulin concentration (Gerozissis et al., 1997, 1998; Orosco et al., 1995). This acute regulation of brain insulin by nutrients shows an anatomical specificity, since the modifications induced in the hypothalamus by a carbohydrate meal are not observed in the cerebellum (Gerozissis et al., 1998). Administration of glucose in the hypothalamus, as an analogy to carbohydrate meals, increases the concentration of insulin in the extracellular space (Gerozissis et al., 2001).

The ingestion and processing of nutrients trigger metabolic and endocrine modifications integrated by the brain, which in turn, *via* a complex multifactorial interaction, control glucoregulation, modulate appetite, further nutrient intake, and energy expenditure, and thus determine body weight and energy balance. Hypothalamic centres detect and integrate the availability of peripheral nutrients *via* redundant and overlapping nutrient-induced peripheral signals such as leptin and insulin and *via* direct metabolic signalling. The arcuate hypothalamic nucleus in particular, has the capacity of detecting directly and responding to nutrients *via* specific metabolic sensing neurons (Lam et al., 2005; Levin, 2006; Wang et al., 2006). Metabolic signals interfere with insulin action. Nutritional signalling controls the transport, production, release or action of interactive with insulin regulators of energy homeostasis, in the hypothalamus. The action of hypothalamic regulators is also sensitive to information transiting through the brain structures that are relays for cognitive events (Fig. 3) (Beck, 1999; Berthoud, 2004; Fehm et al., 2006).

3.2. Interaction of insulin with major neuroregulators

Growing evidence suggests that insulin interacts with both orexigenic and anorexigenic neuromodulators in the brain with regard to the control of feeding behaviour, maintenance of body weight around a set point, energy and glucose homeostasis. Essential nutrients and metabolites participate actively, directly or indirectly, in these multifactorial and multilevel interactions (Fig. 2). In the hypothalamus, there is a complex interplay of insulin with the catabolic factors, [serotonin (5-hydroxytryptamine, 5-HT), leptin, the brain-derived neurotrophic factor, melanocortins], and the anabolic neuropeptides, [neuropeptide Y, agouti related protein (AgRP), melatonin]. Additional

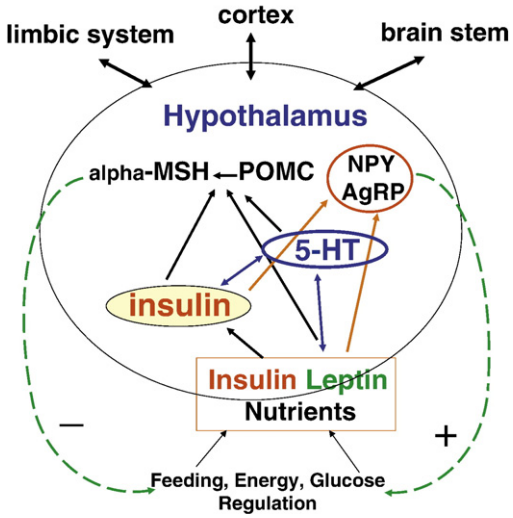

Fig. 3. The hypothalamus senses circulating nutrients, metabolites and hormones, and signals of extrahypothalamic structures, that activate a neurochemical cascade of events. Insulin and leptin downregulate orexigenic peptides such as NPY and AgRP and in interaction with serotonin (5-HT) upregulate melanocortin production and release. These latter peptides mediate most of the anorexigenic effects of insulin, leptin and 5-HT. POMC = Proopiomelanocortin.

complex interactions of insulin and peptides such as galanin, orexins, ghrelin, corticosteroids, melatonin or adiponectin cannot be excluded. Interestingly, many of those peptides can influence insulin release in the pancreas. (For review see Gerozissis et al., 2004).

A growing body of literature suggests that there are multiple possibilities for insulin and leptin interaction in the brain. In addition to a direct dialogue, they may interact *via* other neuromodulators, in particular serotonin (Fig. 3). It has been suggested that the central serotonergic system acts on energy metabolism *via* leptin-responsive hypothalamic pathways (Calapai et al., 1999). In turn, leptin increases serotonin turnover and affects the acute feeding-induced hypothalamic serotonergic stimulation (Telles et al., 2003). We have demonstrated previously bidirectional effects of insulin and serotonin in the median hypothalamus, with serotonin acting immediately on insulin and insulin acting on serotonin with a delay (Orosco et al., 2000). This interaction seems to be a link in a larger cascade of events in the complex regulatory loop between hypothalamic neuromodulators and nutritional behaviour (Fig. 3). The three partners activate proopiomelanocortin neurons, induce an alpha-melanocyte-stimulating hormone production and release, and negatively regulate energy balance (Ahima et al., 2000; Finn et al., 2001; Gerozissis, 2004; Kalra et al., 1999; Orosco and Gerozissis, 2001; Orosco et al., 2000; Zhou et al., 2005).

3.3. Molecular mechanisms of insulin action in the brain

As in peripheral tissue, insulin acts in the brain through its receptors, activating several signalling pathways such as insulin receptor substrate/phosphatidylinositol-3-kinase pathway (IRS/PI3K) and mitogen activated protein kinase (Niswender et al., 2003; Plum et al., 2006). The IRS/PI3K signalling pathway, operating in the hypothalamus, is also activated by leptin, is implicated in food intake regulation and underlines the importance of insulin and leptin receptor cross-talk in the control of energy homeostasis. PI3K activation induces both membrane and genomic effects. (Carvalheira et al., 2001; Harvey et al., 1997, 2000; Niswender and Schwartz, 2003; Niswender et al., 2003, 2004; Porte et al., 2005). Insulin binds to its receptor on proopiomelanocortin and AgRP neurons, stimulating receptor autophosphorylation and activating its signal cascade. IRS proteins bind to the phosphorylated residues on the insulin receptor, recruit the regulatory subunit p85 of PI3K and thus activate PI3K that phosphorylates phosphatidylinositol-4, 5-bisphosphate, generating phosphatidylinositol-3, 4, 5-triphosphate. The protein kinase B/AKT and phosphoinositide-dependent protein kinase 1 (PDK1) bind to phosphatidylinositol-3, 4, 5-triphosphate. The phosphorylated AKT enters the nucleus, where it phosphorylates and inactivates forkhead box protein O1. In proopiomelanocortin neurons, this leads to increased proopiomelanocortin expression. In AgRP neurons, insulin decreases forkhead box protein O1-mediated transcription of AgRP (Plum et al., 2006). Blockade of PI3K activation by i.c.v administration of LY294002 inhibits both insulin and leptin-induced anorexia in rats, assigning to the PI3K pathway a key role in mediating the impact of both hormones on food intake (Niswender et al., 2003, 2004). However, even though insulin and leptin signalling converge at the level of PI3K, the two hormones appear to elicit distinct signalling events downstream of PI3K. Activation of PI3K by leptin and insulin has been demonstrated to differ depending on the cell type: while they act in parallel to stimulate PI3K in proopiomelanocortin neurons, they show opposing effects in AgRP neurons (Xu et al., 2005). Insulin regulates K_{ATP} channel opening by activating PI3K, and proteins downstream in the cascade: PDK1, AKT, glycogen synthase kinase 3 (GSK3), or mammalian target of rapamycin (mTOR). Both insulin and leptin activate hypothalamic K_{ATP} channels, resulting in hyperpolarisation and inactivation of the respective glucose-responsive neurons, a potential mechanism mediating the central effects of insulin and leptin on the modulation of energy homeostasis (Morton et al., 2006; Plum et al., 2006).

Literature suggests that serotonin can activate PI3K in peripheral organs and in the brain (Hsiung et al., 2003; Nebigil et al., 2003). Recently, we demonstrated that the activation of brain PI3K contributes to the inhibition of food intake by serotonin, suggesting that the three partners share the PI3K pathway (Gerozissis et al., 2006, 2007a). Type2c serotonin receptors expressed on proopiomelanocortin neurons are involved in the anorexic effect of serotonin (Heisler et al., 2002). Recent own studies suggest that, further type2 serotonin receptors might be involved in this serotonergic effect (Gerozissis et al., 2006).

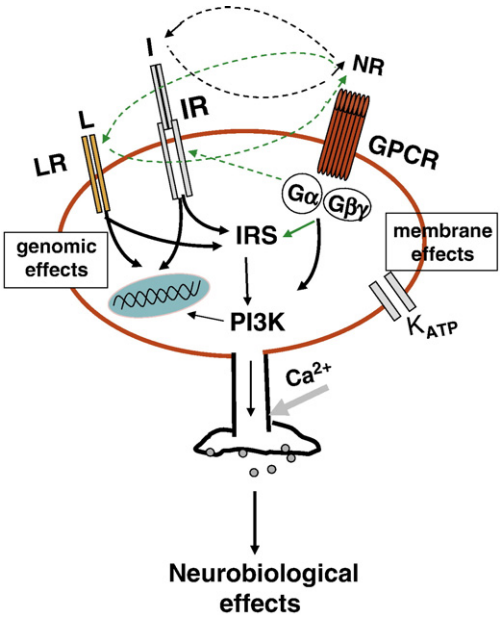

Fig. 4. *Hypothesis*: Insulin (I), leptin (L) and most of neuroregulators (NR) acting through G protein coupling receptors (GPCR), cross-talk directly or indirectly (broken lines) *via* the insulin receptor substrate (IRS)/phosphatidylinositol-3-kinase (PI3K) pathway. We propose that this dialogue is potentially a major event, common to peptides and neurotransmitters, in the pathophysiology of the brain in general and in the regulation of feeding behaviour in particular.

A potential cross-talk involving the PI3K pathway of insulin and additional neuroregulators such as the pineal gland hormone melatonin, or the brain-derived neurotrophic factor, has been proposed. This melatonin and insulin interactive signalling, *via* PI3K, may have a role in the intracellular mechanism controlling body weight, feeding behaviour and blood glucose circadian rhythm. The brain-derived neurotrophic factor receptors can also induce tyrosine phosphorylation on IRS-1, -2, activate PI3K in the brain and induce some similar effects to that of insulin, controlling glucose uptake and metabolism (Mattson et al., 2004; Yamada et al., 1997). Interestingly, brain-derived neurotrophic factor cooperates also with serotonin in biological processes that influence aging and age-related diseases (Mattson et al., 2004). Other hormones that act through G protein-coupled receptors can induce tyrosine phosphorylation. Consequently, it is plausible to suggest that main neuroregulators cross-talk in the brain, *via* either direct or indirect activation of PI3K, and that this dialogue of peptides and neurotransmitters *via* the PI3K pathway, is potentially of major importance in the pathophysiology of the brain in general and specifically in the regulation of feeding behaviour (Fig. 4).

4. The brain insulin and metabolic diseases

4.1. Diminished brain insulin efficiency in obesity and diabetes

Currently, there is a worldwide surge in obesity and type 2 diabetes. According to World Health Organization, the prevalence of diabetes worldwide was projected to increase from 2.8% in 2000 to 4.4% in 2030 and the total number of diabetics was expected to rise from 171 million to 366 million, even if the levels of obesity remain constant. However, given the increasing prevalence of obesity and the link between the two diseases, it is likely that the incidence of diabetes in the future is underestimated (Wild et al., 2004). There has been a dramatic increase observed in juvenile obesity, a condition that greatly increases the risk of persistent obesity and obesity-related health risks during adulthood (Grove and Smith, 2003). While as recently as ten to twenty years ago, juvenile type 2 diabetes was a rare occurrence, at present, it is fast becoming a common medical concern. Consequently, a deepening understanding of the link between obesity and type 2 diabetes is an urgent priority. Over the last ten to fifteen years, important but insufficient progress has been made in understanding the fundamental role of the brain in energy and glucose homeostasis. It has been over 150 years since the French physiologist Claude Bernard, first hypothesized that the central nervous system plays an essential role in the control of peripheral blood glucose levels (Bernard, 1855). Although the French physiologist's ideas were largely ignored, insulin was known to communicate the status of body energy stores to the brain, decades before the discovery of leptin, a hormone that sends similar messages to the brain. The discovery of leptin, introduced a new dimension in the study of the role of the brain in the regulation of energy and glucose homeostasis (Ahima et al., 2000; Fehm et al., 2006; Gerozissis, 2004; Schwartz and Porte, 2005). Absolute insulin deficiency is accompanied by pronounced "diabetic hyperphagia" (Sipols et al., 1995) and defective insulin signalling is a major element linking obesity and diabetes (Lin et al., 2004; Obici et al., 2002a). Mice with neuron-specific insulin receptor deletion show an increase in food intake and body weight (Bruning et al., 2000). The restoration of insulin receptor in the brain of mice with tissue-restricted insulin receptor expression maintains energy homeostasis and prevents diabetes (Okamoto et al., 2004).

As exposed above, in the brain, intact insulin signalling via the IRS/PI3K pathway is essential for energy and glucose homeostasis. Blockade of insulin action in the arcuate nucleus by insulin antibodies, decreasing insulin receptors by antisense oligonucleotides, or inhibiting insulin-dependent activation of PI3K leads to decreased ability of circulating insulin to suppress endogenous glucose production (Prodi and Obici, 2006). A lack of IRS-2 in the hypothalamus results in increased appetite and body mass, leading to insulin resistance and finally diabetes (Lin et al., 2004). Based on genetic studies, Bruning et al. (2000) suggested that insulin resistance both in classical insulin target tissues, and in non-classical target tissues such as the beta cell and the brain, may act synergistically in the induction of obesity, insulin glucose intolerance and dyslipidemia, leading to the complex metabolic syndrome associated with type 2 diabetes.

Recent efforts tempt to understand the common hypothalamic mechanisms involved in insulin and leptin action, in particular the IRS/PI3K pathway. A complex relationship between leptin resistance and insulin resistance occurs at the neuronal level. Actually, cross down-regulation of leptin and insulin receptors at both receptor and downstream signalling levels, including the PI3K activity was observed in a human neuroblastoma cell line (Benomar et al., 2005). Loss-of-function studies are in favour of the hypothesis that impairment of the common part of the pathway is involved in severe pathologies. Defective insulin signalling within key neuronal pathways, in particular in the hypothalamus, along with impaired leptin signalling, can be included among potential mechanisms linking obesity to type 2 diabetes (Lin et al., 2004; Obici et al., 2002a; Porte et al., 2005; Schwartz, 2001; Schwartz and Porte, 2005).

Serotonin, the other important partner of insulin and leptin, is also implicated in metabolic diseases. Literature demonstrates an apparent connection between depressive disorders and diabetes. Several epidemiologic studies confirm that diabetics have increased incidence of depression, and *vice versa*. Patients with clinical depression exhibited a reduced sensitivity to insulin, and insulin efficiency improved with serotonin reuptake inhibitors. In addition, the metabolic syndrome is associated with suppressed neuroendocrine responses to serotonin (Mattson et al., 2004). Literature based on a study of suicide victims, suggests that the PI3K signalling pathway is involved in the serotonergic action in the brain (Hsiung et al., 2003). Interestingly depressive, non-diabetic, patients have several insulin- and glucose-metabolism disturbances (Hundal, 2007).

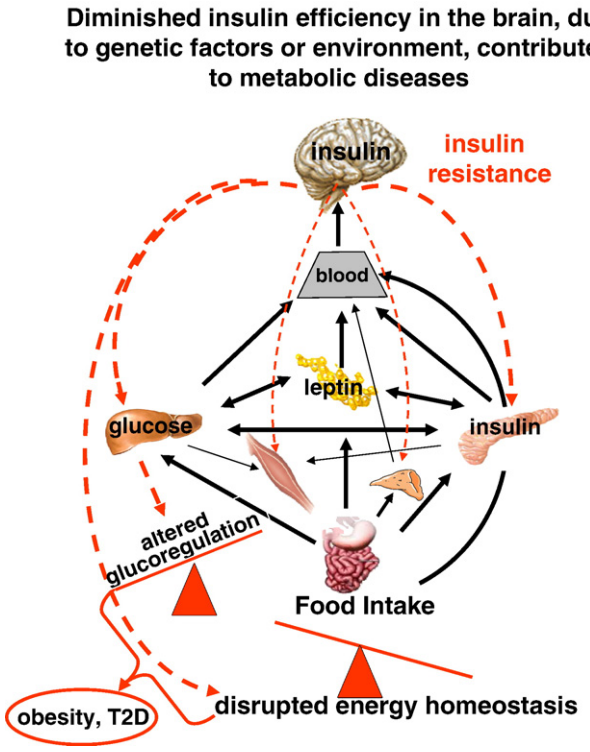

Fig. 5. Alteration of insulin action in the brain contributes to metabolic dysfunctions and severe pathologies. Unbalanced diets associated with further environmental insults and genetic factors, alter the action of insulin in the brain, induce insulin resistance, contribute to the disruption of homeostatic mechanisms and the development of metabolic diseases.

Genetic predisposition, environment and life style, are responsible for the development of metabolic diseases (Fig. 5). Deregulation of insulin secretion and transport in the central nervous system, reduced central to peripheral insulin ratio, along with insulin deficiency or resistance in the brain has been reported in relation to aging, obesity, diabetes and degenerative diseases in *post mortem* studies in humans and in animal models *in vitro* and *in vivo* (Banks, 2004; Frolich et al., 1998; Gerozissis et al., 1993, 2001; Gerozissis 2003; Grunblatt et al., 2007; Kaiyala et al., 2000; Niswender and Schwartz, 2003; Schubert et al., 2004; Spanswick et al., 2000, Steen et al., 2005). Interestingly, some authors consider Alzheimer's disease as the brain type 2 diabetes or as type 3 diabetes (Hoyer, 1998; Steen et al., 2005).

The current surge in the incidence of obesity and diabetes mellitus started some 25–30 years ago. Subsequently, the initiating events that alter the control of the involved homeostatic mechanisms seem to be mostly of environmental origin. However, a genetic predisposition facilitates the initiation of the disturbances. Risk of metabolic disorders is strongly increased by environmental factors that favour weight gain, such as an abundance of unbalanced, energy dense food, combined with low physical activity and stress. High fat diets in particular are incriminated in mechanisms operating in both the periphery and the brain, disrupting energy balance and glucose homeostasis (Woods et al., 2004). An interesting example of increased risk resulting from the association of genetic background and non-genetic factors comes from studies in rats prone to develop diet-induced obesity. This model has reduced central sensitivity to many metabolic and hormonal signals involved in energy homeostasis, in particular in central insulin signalling, which might contribute to its obesity under a high fat diet (Clegg et al., 2005; Irani et al., 2007). High fat diets result in peripheral insulin resistance through an impairment of the ability of insulin to activate the IRS/PI3K pathway (Dresner et al., 1999; Griffin et al., 1999; Niswender and Schwartz, 2003). A similar mechanism leading to combined insulin and leptin resistance during high fat feeding could operate in the hypothalamus. As is the case in animal models, genetically determined and obesity-related cerebral insulin resistance is shown also in humans. Actually, Tschritter et al. (2006), using a magnetoencephalographic study, have observed impaired cortical neuronal response to insulin, in both obese humans and in subjects with genetically reduced insulin signalling at the level of the IRS-1 pathway (Gly972Arg polymorphism in IRS-1), which is considered a type 2 diabetes risk gene. Different patterns of neuronal activity in the two models suggest that central insulin resistance might be not only a consequence but also a starting point for the development of obesity and possibly of type 2 diabetes. The authors hypothesize that in such a case, improvement of brain insulin action may become a therapeutic paradigm to prevent obesity and type 2 diabetes.

The importance of the environment in the early steps of the development of metabolic diseases is in general under investigated. Nevertheless, the few available data suggest a rapid impact of enriched in fat diets on brain insulin. Compared to animals fed a standard laboratory chow diet, rats fed high fat or high energy diets for a short or longer period, show clear differences in the expression of messenger RNA encoding for insulin and modifications of the intracellular insulin signalling pathways, involved in energy metabolism, in the hypothalamus (Gerozissis et al., 2007b; Prada et al., 2005). In addition, unbalanced diets affect brain insulin indirectly by modifying the action of energy regulators associated to brain insulin action. A meal consisting exclusively of animal fat, or a short term high fat diet, both rapidly induce persisting modifications of the serotonergic response to a meal (Banas et al., 2005; Rouch et al., 2005) and attenuate the anorexic effects of a centrally delivered either insulin or a melanocortin receptor agonist (Clegg et al., 2003). Finally, altered responsiveness of the serotonergic system to a meal was recently observed in a model of spontaneous type 2 diabetes that is not overweight, the Goto Kakizaki rat (Gerozissis et al., 2007b). In the same model, messenger RNA encoding for insulin was also modified, showing however a different pattern from that observed in a high fat fed model. These data are in line with the suggestion that brain insulin impairment might be not only a consequence but also a starting point for the development of metabolic dysfunctions (Gerozissis et al., 2007b; Tschritter et al., 2006).

4.2. The brain insulin connection opens new treatment perspectives for metabolic diseases

Current strategies for treating metabolic diseases, in particular type 2 diabetes, are not adequately effective. Most

induce many adverse effects, and probably initiate a vicious cycle of problems (Fehm et al., 2006). There is increasing evidence in favour of complex interactions among the various sites of insulin action and of redundant mechanisms for interorgan communication. These interactions might play a pivotal role in common forms of insulin resistance (Okamoto et al., 2005). Domenico Accili (2004) proposes to replace the concept of diabetes' ruling triumvirate stating that "the combination of impaired insulin-dependent glucose metabolism in skeletal muscle and impaired beta-cell function causes an increase of hepatic glucose production, leading to a constellation of tissue abnormalities". Instead, in place of the triumvirate, he proposes a republic, in which "every tissue contributes to the onset of the disease".

It is well known from epidemiological observations of untreated patients with type 2 diabetes that the increase in body weight is replaced by a decrease upon manifestation of diabetes (Looker et al., 2001). Fehm et al. (2006) suggest that the metabolic syndrome represents the organism's attempt to compensate for the original perturbations on homeostatic mechanisms maintaining a stable body weight. Available therapies for treatment of the metabolic syndrome interfere with mechanisms which must be considered compensatory: these therapies disappoint in the long run. The initiation of insulin therapy in diabetes is usually associated with weight gain (Wing et al., 1990). A strict glucose control is a cornerstone of current treatment of diabetes. However, the results of the United Kingdom Prospective Diabetes Study (UKPDS study group, 1998) demonstrate that strict glycemic control represents a problematic approach for treating diabetic patients: it promotes weight gain when obesity appears to play a fundamental role in the pathophysiology of the disease (Fehm et al., 2006).

There is additional interesting information from animal studies. Male Zucker Diabetic Fatty rats simulate many aspects of human type 2 diabetes, marked by hyperglycemia along with insulin resistance, hyperlipidemia and obesity. At between 7 and 10 weeks of age, Zucker Diabetic Fatty rats develop clinical diabetes and subsequently lose their excess weight. Thiazolidinediones, are agonists of the peroxisome proliferator-activated receptor-γ, enhancing insulin action and thereby improving glycemic control and reducing hyperinsulinemia in both animals and human subjects with type 2 diabetes. These anti-diabetic drugs, like many others, are also known to stimulate food intake and to enhance weight gain in rats. Chronic administration of rosiglitazone, a potent thiazolidinedione, induces morbid obesity to Zucker Diabetic Fatty rats by an as yet unknown mechanism (Cai et al., 2000).

New perspectives for prevention of obesity and diabetes and for insulin therapy in obese people with diabetes come from brain studies (Pocai et al., 2005; Prodi and Obici, 2006; Tschritter et al., 2006). Interestingly, insulin analogues improving glycemic control, without increase of weight gain, increase the efficiency of insulin action in the brain. One of insulin analogues with modified absorption kinetics to improve glycemic control and weight stability, created by amino acid exchange using recombinant DNA technology, has demonstrated a tissue-selective action, with a relative preference for brain compared with peripheral tissues (Hennige et al., 2006). The time course and extent of insulin receptor phosphorylation in peripheral tissues were similar following treatment with the analogue, compared with human insulin, but insulin signalling in the hypothalamus and brain cortex, determined by tyrosine phosphorylation of the insulin receptor and IRS-2 proteins, occurred faster and was enhanced by the analogue. It was suggested that this enhanced brain action, probably due to elevated concentrations of active molecules in the brain, available to bind to the insulin receptor and increase the insulin signalling cascade, might be sufficient to prevent hyperphagia and weight gain in patients.

To reach insulin receptors directly, animals are typically administered insulin *via* the cerebral ventricles. Targeting human brain for diabetic therapy is a particularly challenging problem because of the difficulty to deliver drugs within the brain and maintain therapeutic local levels. Nonetheless, new therapeutic strategies are under active investigation to improve central delivery of drugs (Pardridge, 2007; Prodi and Obici, 2006; Reger and Craft, 2006; Stockhorst et al., 2004). The localization of insulin receptors in the olfactory bulb and indications that central insulin deficiencies are accompanied by olfactory deviations make the nose-to-brain pathway a useful means of insulin administration in the brain (Stockhorst et al., 2004). For humans, the intranasal route for insulin administration is a practicable way to reach the brain while maintaining euglycemia. In fact, the intranasal route, allows direct access to the cerebrospinal fluid compartment within 30 min in a manner that bypasses uptake into the blood stream (Born et al., 2002). Promising initial results have been reported in animals with intranasally administered insulin corresponding to the diverse actions of insulin in the brain. However, studies in humans show that overweight subjects, in particular female patients, are resistant to this treatment (Hallschmid et al., 2004; Fehm et al., 2006). In addition to insulin, regulatory peptides and neurotransmitters, modulators of energy and glucose homeostasis and their receptors, interactive in the central nervous system with insulin, are effective molecular targets for the treatment of metabolic diseases (Prodi and Obici, 2006; Zhou et al., 2005).

5. Conclusion

Nutritional signalling integrated by the hypothalamus, activates a chain of neurochemical events, and triggers interactions between brain structures implicated in feeding behaviour and energy, glucose and fat metabolism. In this cascade of events, insulin has an important implication. Nutrients, metabolic and endocrine factors, together with neural signals, control insulin production and secretion and have a direct impact on circulating insulin levels, on the transport of blood insulin in the brain and on insulin signal transmission. Insulin present in the central nervous system, in concert with anabolic and catabolic neurotransmitters and peptides that are also strongly responsive to nutrients contributes to the short term and long term regulation of essential physiological functions. Of particular interest is the interaction of insulin

with the anorexic brain regulators leptin, serotonin and melanocortins. A specific role in cognitive functions related to feeding is attributed to brain insulin, in interaction with its partners. The activation of the PI3K pathway is an important biochemical step in insulin signal transmission in both the periphery and the brain. Recent studies suggest that this signalling pathway, common to insulin and leptin, is shared also by the neurotransmitter serotonin. We hypothesize that the dialogue of neurotransmitters and peptides *via* this signalling pathway, is potentially a major event in the pathophysiology of brain in general and in the regulation of food intake in particular. A genetic predisposition to metabolic diseases, the abundance of food rich in fat, and low physical activity, disrupt peripheral and central insulin signalling, alter the action of insulin in the brain, induce insulin resistance, and along with leptin resistance, deregulate feeding behaviour and energy homeostasis, and contribute to the onset of obesity to diabetes. Most of the current strategies for prevention and treating of metabolic diseases, in particular type 2 diabetes, do not take into account the multifactorial, complex interactions among the various sites of insulin action and they are not satisfactory. Therefore there is an urgent need to consider the importance and the complexity of insulin efficiency in every tissue and in particular in the brain. A complete understanding of the molecular mechanisms involved in the action of insulin in the central nervous system, in both hypothalamic and extrahypothalamic areas, will clarify an important aspect of the pathophysiology of energy and glucose homeostasis. Further understanding of the interaction of central and peripheral levels of the effects of insulin, and its cross-talk with its numerous partners, will, we hope, facilitate the design of more efficient preventive strategies, drug development with limited side effects and help improving our therapeutic approaches for obesity and diabetes.

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