Is insulin resistance a disorder of the brain?

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1. ABSTRACT

There is reasonable evidence to suggest that insulin resistance may have its origins in the hypothalamus. Insulin secretion is regulated by sympathetic and parasympathetic nervous systems and modulates the concentrations of hypothalamic neuropeptides and monoaminergic neurotransmitters, and, in return, hypothalamic monoamines regulate the secretion of insulin by pancreatic beta cells. A lesion of the ventromedial hypothalamus produces all the features of the metabolic syndrome including insulin resistance and hyperinsulinemia. These and other evidence suggest that insulin resistance may very well be a disease of the brain.

2. INTRODUCTION

Peripheral insulin resistance is common in obesity, type 2 diabetes mellitus, coronary heart disease (CHD), and hyperlipidemia. There is evidence to suggest that some well defined hypothalamic neurons may have a significant role both in insulin secretion and resistance. The main brainstem parasympathetic efferent neurons reach the pancreas and regulate insulin secretion (Figure 1). These parasympathetic efferent neurons are located in the nucleus ambiguous and the dorsal motor nucleus of the vagus nerve. Ventromedial hypothalamic (VMH) lesions produce insulin overseretion an abnormality that seems to be vagus nerve-mediated. VMH lesions result in an increased parasympathetic efferent tone together with decreased
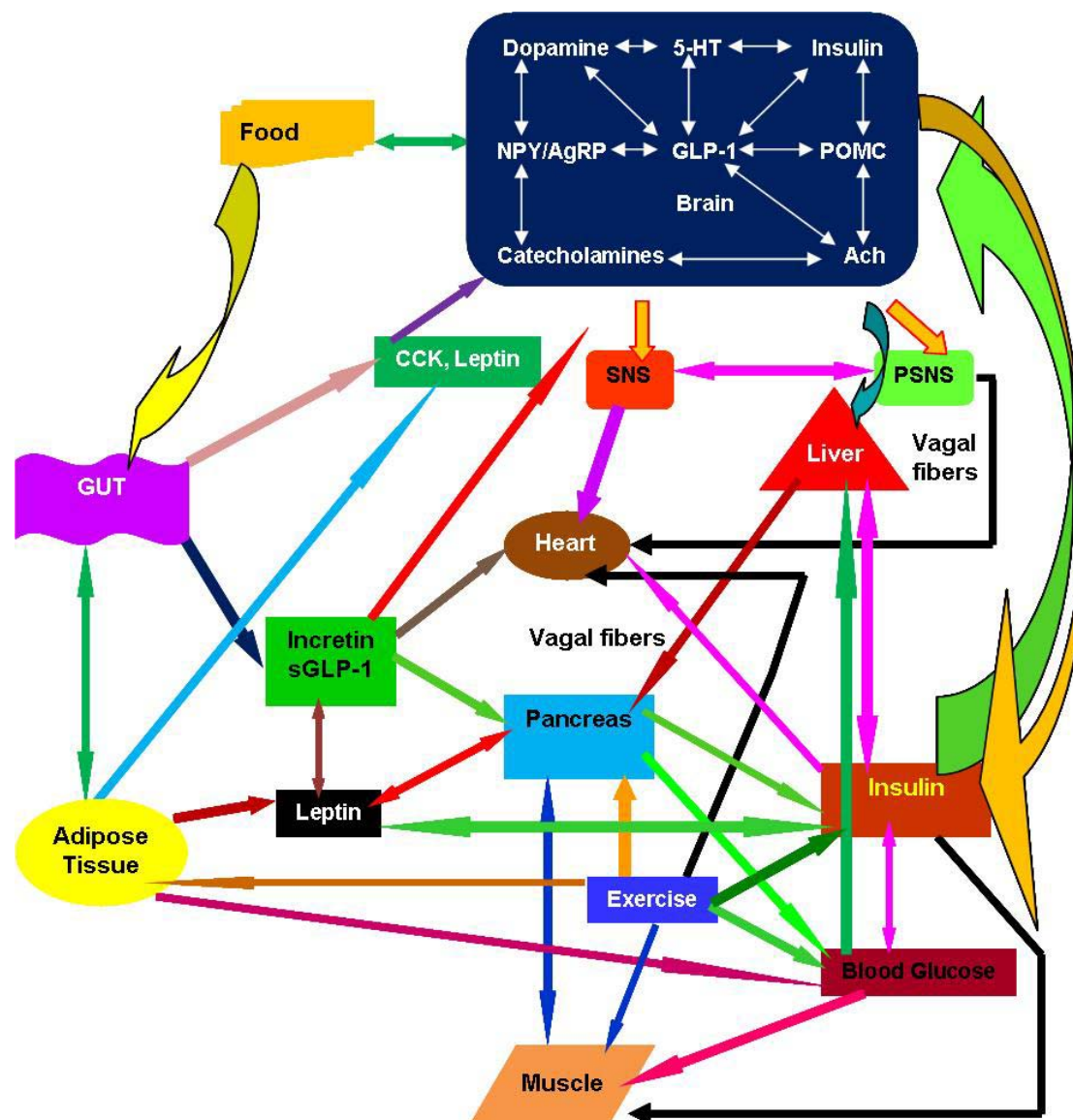
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Figure 1. Scheme showing relationship among hypothalamic neuropeptides, monoaminergic transmitters, gut, leptin and other molecules; pancreas, liver, muscle, adipose tissue and exercise. Hypothalamus and other areas of brain are rich in insulin receptors and GLP-1 receptors. Thus, it is likely that GLP-1 (though not discussed in details in the present article) secreted by the gut could reach brain and regulate insulin secretion by its actions on the hypothalamus in addition to acting directly on pancreatic beta cells to increase insulin secretion. GLP-1 receptors are also situated on the heart and thus may have cardioprotective action. Leptin and insulin interact with each other. Insulin receptor number and their affinity to insulin in the hypothalamus is one significant factor that has a regulatory action on the development of obesity, type 2 diabetes mellitus and the metabolic syndrome as evident from studies done with NIRKO mice. Insulin could regulate the secretion and action of NPY/Agrp, dopamine, serotonin and Ach in the brain. Ach, in turn, has a regulatory action on serotonin and dopamine and catecholamines. Normally there is a balance between sympathetic (SNS) and parasympathetic nervous systems (PNS). PNS by virtue of its Ach secretion could regulate inflammation, NO production, cardiovascular system, insulin secretion and homeostatic functions of liver. GLP-1 receptors are present in the brain that may have a regulatory role in insulin action. Brain communicates with liver through vagal efferent fibers and liver, in turn, communicated with pancreas through vagal fibers. There are also vagal afferent and efferent fibers running between hypothalamus and pancreas that control insulin secretion. Exercise enhanced vagal tone that may be relevant to its beneficial actions on the regulation blood glucose control, prevention of cardiovascular diseases and inflammation. Leptin receptors are also present on pancreatic beta cells and thus, may have a regulatory role in insulin secretion. In summary, brain (hypothalamus), gut, pancreas, adipose tissue, heart, muscle, liver and autonomic nervous system behave as one integrated wheel in the regulation of glucose homeostasis.

sympathetic tone that leads to hyperinsulinemia, obesity and insulin resistance (1).

3. PARASYMPATHETIC AND SYMPATHETIC TONES AND INSULIN RESISTANCE

In experimental animals, glucose-induced insulin secretion was greater in pre-weaned pre-obese 17-day-old Zucker rats compared to the corresponding controls that were reversed to normal by pretreatment with atropine. It is interesting to note that 30 second electrical stimulation of the vagus nerve (that induces the release of acetylcholine) preceding a glucose load potentiated the glucose load-induced insulin release in 6-9 week adult animals and more so in obese Zucker (fa/fa) than in lean rats that supports the contention that there exists an enhanced sensitivity and/or responsiveness of the pancreatic beta cells of obese animals to the parasympathetic system. This was confirmed by the observation that vagotomy resulted in a significant decrease in glucose-induced insulin secretion, an effect that was absent in lean rats. Perfusion of pancreas from adult obese Zucker rats showed enhanced secretion of insulin in response to arginine when compared with controls, an effect that was restored to normal by superimposed atropine infusion suggesting that arginine enhanced vagal tone, possibly, by augmenting the synthesis of nitric oxide. These results suggest that enhanced insulin secretion in pre-obese Zucker rats is an early abnormality that is mediated by increased tone of the parasympathetic vagus nerve and that in adult obese rats increased insulin secretion is also vagus-mediated (2, 3).

In the genetically obese rat (fa/fa), wherein an *in situ* brain-pancreas perfusion model with intact pancreatic central nervous system (CNS) innervations model was used, it was noted that though the overall pattern of insulin secretory dynamics were similar in both the obese and lean rats, insulin released during the entire 40 minute perfusion period from obese rats was significantly greater than in lean rats. Obese rats with intact CNS secreted almost 2-fold higher amounts of insulin compared with the obese in which the CNS was ablated and 4-fold higher amounts of insulin as CNS intact lean rats, suggesting that hypersecretion of insulin in obese Zucker rats is controlled by CNS. Furthermore, vagotomy had little effect on CNS intact lean rats whereas it reversed the CNS component of hypersecretion of insulin in the CNS intact obese rats but insulin secretion was lowered by vagotomy (4). These results suggest that parasympathetic nervous system plays a significant role in the hyperinsulinemia seen in the Zucker obese rat that could be due to the direct innervations of the pancreas by parasympathetic nerves.

Under normal physiological conditions, a balance is maintained between parasympathetic and sympathetic nervous systems and their tone. Hence, it is possible that the enhanced tone of the vagus nerve noted in pre-obese Zucker rats and obese adult rats could be accompanied by a simultaneous decrease in sympathetic tone. This is supported by the observation that hypersecretion of insulin in obese rats was partially due to diminished tonic sympathetic nervous system inhibition of insulin release (5)

providing evidence that abnormal CNS control of insulin secretion in obese Zucker rats.

4. HYPOTHALAMO-PITUITARY-ADRENAL PATHWAY AND PARASYMPATHETIC AND SYMPATHETIC SYSTEMS AND HYPOTHALAMIC NEUROPEPTIDE Y IN INSULIN RESISTANCE

It is known that (a) pre-weaning adrenalectomy prevented the development of hyperinsulinemia in genetically obese (fa/fa) rats, (b) corticosterone replacement for only 24 h restored the hyperinsulinemia of obese (fa/fa) rats, (c) the differential effects of corticosterone on insulin secretion by lean and obese rats were found to be mediated by the parasympathetic nervous system and (d) the parasympathetic nervous system contributes to, but is not the only cause of, hyperinsulinemia in intact obese rats, although plasma glucose concentrations did not differ between phenotypes (6). The basal plasma insulin concentration of obese rats treated with corticosterone for 24 h was reduced 15, 30 and 45 min after injection of atropine (0.3 mg) without any change in the plasma glucose level suggesting that even corticosteroid-induced hyperinsulinemia is mediated to some extent by the parasympathetic nervous system. Furthermore, the insulin secretory response to the glucose load was greater in obese than in lean rats that was significantly reduced by atropine in obese (7). Adrenalectomy not only reduced basal insulin levels and the secretory response in obese but not lean rats but also abolished the atropine-blockable component of the response, whereas corticosterone replacement of adrenalectomized fa/fa rats restored the hyperinsulinemia. Infusion of dexamethasone intracerebro-ventricularly to adrenalectomized fa/fa rats increased basal insulin and the secretory response to glucose, an effect that was blocked by atropine. In contrast, intracerebroventricular infusion of obese rats with corticotropin releasing factor reduced basal and stimulated insulin levels. These results reiterate the fact that the hypersecretion of insulin in obese fa/fa rats are mediated by the central glucocorticoid-mediated stimulation of vagal drive to the pancreatic beta-cells implicating the hypothalamo-pituitary-adrenal (HPA) axis in the pathogenesis of obesity.

Administration of an ovine corticotropin-releasing factor (oCRF) bolus that does not stimulate HPA axis at the onset of glucose ingestion during oral glucose tolerance tests (OGTTs) normalized the glucose intolerance of genetically obese rats and decreased their insulin output, whereas it had no effect in lean rats, and did not change glucose absorption. This indicates that the beneficial effect of oCRF on glucose intolerance of fa/fa rats was not dependent on glucose absorption (8). In contrast, when the intravenous bolus of oCRF was doubled at the onset of OGTTs, it stimulated the HPA axis and produced a worsening of glucose intolerance in obese rats together with an increase in their insulin response but had no effect in lean rats. These results coupled with the observations that: (a) the white adipose tissue lipogenic activity was much more insulin responsive whereas the muscle tissue

was insulin resistant in normal rats exposed to hyperinsulinemia for 4 days than in the control groups with corresponding and divergent changes in glucose transporter (GLUT-4); (b) muscle tissue of normal rats exposed to stress levels of corticosterone for 2 days showed insulin resistance due to an increased glucose-fatty acid cycle, without measurable alteration of the GLUT-4; (c) genetically obese (fa/fa) rats showed decreased cerebral glucose utilization compared to lean controls due to increased levels in their hypothalamic neuropeptide Y (NPY) levels and median eminence corticotropin-releasing-factor; and (d) Intracerebro-ventricular administration of NPY to normal rats for 7 days produced hyperinsulinemia, hypercorticosteronaemia, and other metabolic abnormalities seen in the genetically obese fa/fa rats, including muscle insulin resistance, suggested that both obesity and insulin resistance could be disorder(s) of the brain (9).

5. NPY, LEPTIN, GLUT-4, MELANOCORTIN, AND INSULIN INTERACT TO PLAY A ROLE IN INSULIN RESISTANCE

Genetically obese rodents that showed hyperphagia, hyperinsulinemia, and insulin resistance has increased hypothalamic NPY mRNA and peptide content (10). When normal rats were given intracerebroventricularly NPY, they showed: (a) marked hyperphagia, (b) increased body weight gain, (c) increased basal insulinemia, (d) a much greater insulin response to meal feeding, (e) a pronounced increase in the *in vivo* insulin-stimulated glucose uptake by adipose tissue with a marked decrease in uptake by muscle tissue, (f) increased insulin responsiveness of the glucose transport process by adipose tissue due to increases in both GLUT4 mRNA and protein levels, and (g) decreased insulin responsiveness of glucose uptake in muscles unrelated to GLUT-4 expression compared with saline-infused controls implicating NPY as a major player in the development of insulin resistance and the metabolic syndrome. Intracerebroventricular administration of NPY paralleled increase in ob gene expression in adipose tissue. When normal rats were made hyperinsulinemic-euglycaemic for 24 hours, such hyperinsulinemia also resulted in increased ob mRNA levels in white adipose tissue (11). Thus, NPY-induced hyperinsulinemia seems to be responsible for the upregulation of ob mRNA levels of NPY-infused rats and hence, a functional relationship is likely between NPY-induced hyperinsulinemia and ob gene expression in the adipose tissue. It should be mentioned here that increased hypothalamic (NPY action and disruption of the melanocortin (MC)-4 receptor (MC4 is a satiety factor) both result in hyperphagia and obesity but MC4 receptor antagonism does not induce obesity by regulating the endogenous NPY-ergic system (12, 13). Blockade of the MC4 receptor induced obesity with no apparent side-effects suggesting that MC4 receptor blockade does not produce hyperphagia by increased NPY release, because hypothalamic NPY gene expression was markedly reduced, suggesting that hyperphagia mainly resulted from loss of the satiety signal driven by MC peptides. NPY infusion produced hypogonadism and hyposomatotropism in the

face of markedly elevated plasma leptin levels and a reduction in hypothalamic POMC (pro-opiomelanocortin) synthesis, implying that NPY acts both by exacerbating food intake through Y receptors and by reducing the satiety signal driven by MC peptides.

POMC, the precursor of adrenocorticotrophin hormone (ACTH), melanocortin stimulating hormone (MSH), and beta-endorphin peptides, is expressed in the pituitary, the arcuate nucleus of the hypothalamus and the commissural nucleus of the solitary tract of the brain stem. The melanocortin 3 receptor (MC3-R) was found in arcuate nucleus neurons and in the brainstem. In contrast, MC4-R was found in multiple sites in virtually every brain region, including the cortex, thalamus, hypothalamus, brainstem, and spinal cord. MC4-R mRNA is found in both parvicellular and magnocellular neurons of the paraventricular nucleus of the hypothalamus, suggesting that it may have a role in the control of pituitary function. MC4-R is also expressed in numerous cortical and brainstem nuclei. Together, MC3-R and/or MC4-R mRNA are found in every nucleus reported to bind MSH in the adult rat brain and define neuronal circuitry known to be involved in the control of autonomic functions (14). The POMC-derived peptides are important regulators of food intake and metabolic and autonomic responses.

Intracerebroventricular administration of alpha-MSH and ACTH resulted in a significant increase in the lumbar sympathetic nerve activity (LSNA) that was accompanied by an increase in mean arterial pressure (MAP), while the administration of beta-endorphin decreased the LSNA and MAP. MC4 receptor antagonist reverses the endorphin response and the opioid antagonist attenuates the alpha-MSH response suggesting interactions between MC4 and the opioid receptor mediated effects (15). In addition, melanocortin system mediates the central effects of leptin and promotes fat deposition via both food intake-dependent and -independent mechanisms (16). The hypothalamic melanocortin system increases sympathetic nerve traffic to thermogenic brown adipose tissue (BAT) and other tissues. Leptin increases renal sympathetic nerve activity through activation of hypothalamic melanocortin receptors, while sympathetic activation to thermogenic brown adipose tissue by leptin is independent of the melanocortin system (17). Administration of the MC receptor antagonist SHU9119 to rats for 11 days doubled food and water intake and increased body weight (~ 14%) and fat content (~ 90%), hepatic glycogen content (~ 40%), and plasma levels of cholesterol (~ 48%), insulin (~ 259%), glucagon (~ 80%), and leptin (~ 490%), whereas spontaneous locomotor activity and body temperature were reduced. Pair-feeding of third intracerebroventricular (i3vt) administration SHU9119-treated animals to i3vt vehicle-treated controls normalized plasma levels of insulin, glucagon, and hepatic glycogen content, but only partially reversed the elevations of plasma cholesterol and leptin and body fat content and did not show reductions in body temperature and locomotor activity induced by i3vt SHU9119 and in fact, were more pronounced. In the MC receptor antagonist treated animals obesity effects occurred despite a lack of change in the expression of in

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neuropeptides: CART, POMC, and NPY in the arcuate and of CRH in the paraventricular hypothalamus. These results suggest that reduced activity of the CNS MC pathway could produce all the manifestation of the metabolic syndrome. Hyperleptinemia also produced elevation of blood pressure and increased urinary catecholamine excretion that was abolished by alpha$_1$-adrenergic, beta-adrenergic, or ganglionic blockers suggesting that leptin stimulates sympathetic nervous system (18).

In normal animals leptin increases pro-opiomelanocortin (POMC) expression and especially the increase in alpha-MSH, whereas high fat diet induced obese animals show a decreased sensitivity to alpha-MSH that altered leptin resistance characteristic of obese animals (19). The co-existence of hyperinsulinemia and hyperleptinemia seen in obesity in which both insulin resistance and leptin resistance are common suggests a close interaction between insulin and leptin. Leptin receptors are located on hypothalamic neurons that co-express NPY or POMC and both these peptides are implicated as mediators of the CNS action of leptin. Leptin decreases or down regulates NPY expression and increases POMC expression. Insulin also decreases NPY expression and insulin insufficiency is associated with an increased POMC. Since both leptin and insulin share and modulate the same effector systems, it is likely that they interact with each other. When normal rats were pre-treated intracerebroventricularly (ICV) with insulin for 3 days, ICV leptin administration-induced increase in both lumbar sympathetic nerve activity and mean arterial pressure were completely attenuated. However, insulin treatment did not affect the POMC peptide product alpha-MSH hormone mediated sympathetic nervous and cardiovascular responses. These results suggest that CNS hyperinsulinemia blocks leptin-induced increases in sympathetic nervous and cardiovascular system activity and hyperinsulinemia of obesity has a role in the obesity-induced leptin resistance (20).

6. INSULIN AND BRAIN

It is evident from the preceding discussion that hypothalamus regulates many, if not all, actions of insulin. Several other studies do suggest that the actions of insulin on specific areas of brain are critical to the secretion of insulin by pancreatic beta cells and the development of peripheral insulin resistance. In a study (21) performed to known whether genetically obese Zucker rats present changes in brain glucose utilization and/or insulin binding when compared to their lean counterparts, it was noted that glucose utilization in the whole brain was significantly lower in obese than in lean Zucker rats. It was noted that in obese rats, local cerebral glucose utilization (LCGU) was significantly decreased in the external plexiform layer (-37%, p < 0.05), in the lateral hypothalamus (-23%, p < 0.05), and in the basolateral amygdaloid nucleus (-30%, p < 0.05) whereas no difference in specific insulin binding was found in any of the areas studied. These results suggest that glucose utilization is defective in hyperinsulinemic rats that may have relevance to the regulation of body weight and food intake in obesity. In contrast, insulin binding in the

arcuate (ARC), dorsomedial (DN), and ventromedial (VMN) hypothalamic nuclei of 3-4 month old lean (Fa/Fa) and genetically obese (fa/fa) Zucker rats a 15% increase in the total specific binding of ^{125}I-insulin in the ARC of the obese genotype and a much less increase in insulin binding in the DMN was noted (22). It was reported that the ARC insulin binding site was 33% higher in the lean rats than in the obese rats, indicating an increased affinity for insulin suggesting that hyperphagia and obesity of the obese (fa/fa) Zucker rat genotype may be associated with increased insulin binding in the arcuate nucleus.

In this context, it is important to note that administration of intracerebroventricular insulin significantly suppressed fasting induced increased expression of pre-pro-NPY mRNA in the arcuate nucleus, immunoreactive NPY concentrations in the paraventricular nucleus of Long-Evans and Wistar rats without affecting plasma glucose or insulin levels. These results indicated that insulin acts locally on hypothalamus to suppress NPY production and thus, regulates feeding behavior (23, 24). On the other hand, central insulin administration of insulin to obese Zucker rats after food privation showed that insulin suppresses the expression of NPY in the ARC of fasted lean but not obese Zucker rats. Thus, regulation of hypothalamic NPY gene expression could be one of the main actions of insulin that accounts for its anorexiant effect, and a defect in this action may contribute to obesity. These results coupled with the observation that a 2- to 3-fold increase in pre-pro-NPY mRNA levels in the arcuate nucleus of the hypothalamus of obese animals occurred compared to lean. A 72 hour food deprivation lead to a 2-fold increase in pre-pro-NPY mRNA content in the lean animals, whereas obese animals showed no such increase after food deprivation (25). These data support the idea that hypothalamic NPY is involved in regulating feeding behavior and weight gain, and that disturbed regulation of hypothalamic NPY expression, possibly due to insulin, plays a role in the etiology of obesity. Thus, there exists a feedback regulation between NPY and insulin in the brain: by acting on the hypothalamic arcuate nucleus in the brain, insulin suppresses food intake, whereas neuropeptide Y (NPY) has the opposite effect. Fasting increases NPY gene expression in the hypothalamic arcuate nucleus (ARC) and also lowers circulating insulin levels, whereas after feeding NPY levels are decreased whereas insulin levels increase in the arcuate nucleus to inhibit food intake and induce satiety. This positive and negative feedback regulation between NPY and insulin in the hypothalamic arcuate nucleus is defective or lost in obesity. Furthermore, the expression of pre-pro-NPY mRNA remains significantly elevated in obese animals even during fasting compared to control lean animals despite normal and/or lower plasma insulin levels indicating a mismatch between insulin and NPY levels and their actions.

The role of insulin and its receptors (IRs) in the brain in the pathogenesis of insulin resistance, obesity and the metabolic syndrome is evident from the observation that mice with a neuron-specific disruption of the IR gene (NIRKO mice), especially female mice, showed increased food intake, and both male and female mice developed diet-

sensitive obesity with increases in body fat and plasma leptin levels, mild insulin resistance, hyperinsulinemia, enhanced plasma insulin levels, and hypertriglyceridemia with normal brain development and neuronal survival. NIRKO mice also showed impaired spermatogenesis and ovarian follicle maturation because of hypothalamic dysregulation of luteinizing hormone (26). In contrast to this, mice with fat-specific disruption of the insulin receptor gene (FIRKO mice) have low fat mass, loss of the normal relationship between plasma leptin and body weight, and are protected against age-related and hypothalamic lesion-induced obesity, and obesity-related glucose intolerance. FIRKO mice exhibited polarization of adipocytes into populations of large and small cells, which differ in expression of fatty acid synthase, C/EBP alpha, and SREBP-1. These results imply that insulin signaling in adipocytes is critical for development of obesity and its associated metabolic abnormalities (27).

In addition, selective decreases in insulin receptor expression in the medial portion of the arcuate nucleus (decreased by approximately 80% as compared to rats treated with a control) exhibited rapid onset of hyperphagia and increased fat mass, and hyperinsulinemia (28). Thus, insulin receptors in the hypothalamic arcuate nucleus have a distinct physiological role in the control of food intake, fat mass, hepatic action of insulin, and development of insulin resistance. Insulin-induced tyrosine phosphorylation of the insulin receptor and SRC homology adaptor protein (SHC), and the association of SHC/growth factor receptor binding protein-2 (GRB2) decreased significantly from day 1 to week 60 of life in the forebrain cortex and cerebellum. With ageing, the expression of SH protein tyrosine phosphatase-2 (SHP2), a tyrosine phosphatase involved in insulin signal transduction and regulation of the insulin signal, decreased significantly in both the forebrain cortex and the cerebellum of rats (29). Thus, insulin-signaling pathway is dysfunctional in the aged brain that may explain as to why development of insulin resistance and type 2 diabetes mellitus is common with advancing age.

The role of brain in insulin resistance is further supported by the observation that intracerebroventricular insulin infusion reduced food intake in lean rats to a greater extent than that observed in obese rats, and pre-treatment with PI3-kinase inhibitors prevented insulin-induced anorexia. Insulin-stimulated phosphorylation of IR, IRS-1/2, the associations of PI 3-kinase to IRS-1/2 and phosphorylation of Akt in hypothalamus were decreased in obese rats compared to lean rats. In contrast, insulin stimulated the phosphorylation of MAP kinase equally in lean and obese rats. These results provide support for the hypothesis that the anti-obesity actions of insulin are mediated by the PI3-kinase pathway, and that impaired insulin signaling in hypothalamus plays a role in the development of obesity in obese Zucker rats that have insulin-resistance (30).

It was reported that *ex vivo*, insulin-stimulated tyrosine phosphorylation of insulin receptor beta subunits (IR-beta) in the aorta and microvessels of obese rats was significantly decreased compared with lean rats, although the protein levels of IR-beta in the 2 groups were equal. Insulin-induced tyrosine phosphorylation of insulin receptor substrates 1 and 2 (IRS-1 and IRS-2) and their protein levels were decreased in the aorta of obese rats compared with lean rats. The association of p85 subunit to the IRS proteins and the IRS-associated PI 3-kinase activities stimulated by insulin in the aorta of obese rats were significantly decreased compared with the lean rats. Insulin-stimulated serine phosphorylation of Akt, a downstream kinase of PI 3-kinase pathway, was also reduced significantly in isolated microvessels from obese rats compared with the lean rats. In euglycaemic clamp studies, insulin infusion greatly increased tyrosine phosphorylation of IR-beta- and IRS-2-associated PI 3-kinase activity in the aorta of lean rats, but only slight increase or no change were observed in obese rats. In contrast, insulin stimulated tyrosine phosphorylation of MAP kinase (ERK-1/2) equally in isolated microvessels of lean and obese rats, although basal tyrosine phosphorylation of ERK-1/2 was higher in the obese rats. These data suggested that a selective resistance to PI 3-kinase (but not to MAP kinase pathway) occurs in the vascular tissues of obese Zucker rats (31). Thus, insulin mediated PI3-kinase pathway is defective both in the hypothalamus and vascular tissue in obesity.

Since chronic hyperinsulinemic rats showed insulin resistance and reduced levels of glycogen content in liver and muscle; impairment of the insulin-induced IR/IRSs/PI3K/Akt pathway in liver and muscle that parallels increases in IRS1/2 serine phosphorylation, IR/PTP1B association and mTOR activity; an increase in IRS-1/2 protein levels, tyrosine phosphorylation and IRSs/PI3K association, and an increase in basal Akt serine phosphorylation in white adipose tissue that were reversed by rapamycin treatment in the liver, muscle and white adipose tissue provides further evidence that defective tissue-selective insulin action contributes to the insulin resistance observed in obesity (32). Thus, defects in insulin-stimulated phosphorylation of IR, IRS-1/2, the associations of PI 3-kinase to IRS-1/2 and phosphorylation of Akt is a generalized phenomena in most of the insulin target tissues (muscle, liver, brain and vascular tissues) in obesity that may be responsible for insulin resistance though the degree of resistance and which tissue is the first to develop resistance is debatable. It is likely that adipose tissue shows the maximum insulin resistance (adipose > liver > muscle > hypothalamus) and the first tissue to develop insulin resistance could be the hypothalamic neurons (the sequence could be as follows: first hypothalamic neurons followed by muscle, then the liver and lastly the adipose tissue).

7. INSULIN AND BRAIN MONOAMINES

Fasting, hypoglycemia and streptozotocin-induced diabetes have been shown to influence brain tryptophan and serotonin metabolism (33-35). In the genetically obese yellow (Ay/a) mice at the age of 6 weeks when there was no difference in body weight between black (a/a) and yellow (Ay/a) mice, the norepinephrine

(NE), dopamine (DA) and their main metabolites (MHPG, DOPAC = 3, 4-dihydroxyphenylacetic acid) contents were significantly reduced in yellow (Ay/a) mice. Reduction of 5-hydroxytryptamine (5-HT) level and an increasing 5-HIAA/5-HT ratio was noted. At the age of 12 weeks, when a significant increase in body weight in the yellow (Ay/a) mice was present, both NE and DA contents were increased in the hypothalamus of the obese mouse, whereas MHPG level was lower than in the lean mouse, resulting in an increase of MHPG/NE ratio. These results suggested that a reduction in hypothalamic NE and DA metabolism might be involved in the development of overweight gain in the yellow (Ay/a) mouse (36). Similar changes in the hypothalamic DA and NE contents, viz. they were significantly reduced, and the DOPAC/DA ratio was significantly increased in the hypothalamus of obese rats (37).

It was reported that serum norepinephrine and epinephrine increased following injection of insulin, and food intake rose after a delay of 30-60 min. Norepinephrine rose in both the ventromedial and lateral hypothalamus. 3-Methoxy-4-hydroxy-phenylglycol (MHPG) and 3, 4-dihydroxyphenylacetic acid (DOPAC) increased in the lateral but not the ventromedial hypothalamus. Serotonin was unchanged following the injection of insulin, but its metabolite, 5-hydroxy-3-indole acetic acid (5-HIAA), fell gradually in both ventromedial and lateral hypothalamic areas. Animals that had recovered from the hypoglycemia during the 6 h after insulin injection, but had not been allowed to eat, the concentration of norepinephrine, serotonin, MHPG and 5-HIAA were all increased in the lateral hypothalamus with no change in DOPAC. All monoamines and their metabolites fell to or toward normal within 30-60 minutes after the initiation of food intake (38). These data support the proposal that hypoglycemia increases turnover of norepinephrine and serotonin. Furthermore, streptozotocin-diabetic rats possess a reduced striatal dopamine metabolism that is counteracted by insulin administration (39). A blunted VMN serotonergic activity was noted in genetically obese Zucker rats implying a significant role for serotonin in the development of obesity (40).

Even in alloxan-induced diabetic animal model, an increase in noradrenaline level in the anterior and the medial-basal hypothalamus and a concomitant rise in dopamine content in the hypothalamus, a fall in serotonin level in all the parts of hypothalamus in prediabetic animals have been reported, suggesting that there are significant alterations in the hypothalamic monoamines. It was reported (41) that the contents of 5-hydroxyindoleacetic acid and homovanillic acid, in the whole brain gradually decreased with the duration of diabetes, an increase of norepinephrine content and dopamine contents were found in the hypothalamus. It was noted that diabetic patients had an increase in the content of serotonin in the medial and lateral hypothalamus, increased dopamine in the medial hypothalamus, putamen, and medial and lateral pallidus, increased norepinephrine in the lateral pallidus and decreased in the nucleus accumbens and claustrum. These studies performed in human brain post mortem confirmed that the changes in the brain monoamines are similar both in diabetic rats and diabetic patients.

In normal male Wistar rats during the cephalic phase of insulin secretion, there were increased levels of norepinephrine, its metabolite dihydroxyphenylethylene glycol (DHPG), and serotonin in the lateral hypothalamus and serotonin, its metabolite 5-hydroxyindoleacetic acid, and the dopamine metabolite DOPAC were all higher in the VMH (ventromedial hypothalamus) despite the fact there was no change in the plasma glucose levels both in the control and test group though experimental animals had significantly higher serum insulin levels than did the control animals. Serotonin levels in the LH and DOPAC levels in the VMH were closely associated with serum insulin. Thus, changes in the monoamines in specific areas of the hypothalamus are associated with the cephalic phase insulin release (42, 43). These results support the proposal that hypothalamic monoamines control the release of insulin from the pancreatic beta cells.

The observation that infusion of norepinephrine (25 nmol/hour) and serotonin (2.5 nmol/hour) into the VMH of normal hamsters for 5 weeks produced a 2-6 fold increase in glucose-induced insulin release suggested that an increase of noradrenaline and serotonin content in the VMH can induce dysregulation of islet insulin release in response to glucose and neurotransmitters (44). In addition, hyperinsulinemic and insulin-resistant animals showed elevated levels of noradrenaline and serotonin in VMH (45), indicating that an endogenous increase in these hypothalamic monoamines may contribute to islet dysfunction, which is one of the characteristics of type 2 diabetes.

Since insulin and insulin receptors are present in the mammalian brain, it reasonable to assume that an interaction between monoamines and insulin in the brain could exist. One of the main functions of insulin is to regulate glucose homeostasis. Hence, it is likely that insulin and insulin receptors present in the brain modulate glucose metabolism not only in the brain but also in the peripheral tissues. Since noradrenaline, serotonin and dopamine levels in the hypothalamus are altered in obesity and diabetes mellitus, it is likely that insulin may have an effect on these monoamines.

Intracerebroventricularly (ICV) administered insulin in doses which induced minimal hypoglycemia, dopamine in midbrain-diencephalon and caudate nucleus (CN), noradrenaline and serotonin in midbrain and pons-medulla (PM), were more in the hyperglycaemic rats as compared to their euglycaemic counterparts, whereas those of acetylcholine were lower in these three areas. Insulin induced a decrease in rat brain dopamine and noradrenaline levels, which was more marked in the hyperglycaemic animals; and an insignificant increase in brain serotonin concentration. Insulin induced marked increase in rat brain acetylcholine levels, which was accentuated in hyperglycaemic animals. These studies suggested that an interaction exists between brain insulin receptors and brain

monoamines, and acetylcholine in euglycaemic and hyperglycaemic states (46) and that insulin brings about some of its actions by modulating the levels of various monoamines in the brain. These results suggest that dynamic changes occur in various hypothalamic nuclei especially in the VMH and in the paraventricular nucleus (PVN) monoamines in association with plasma glucose and insulin levels that correspond to fasting and feeding.

Both in the genetically obese Zucker and normal rats, serotonin, 5-HIAA and dopamine increased at the beginning of spontaneous meals while DOPAC decreased except that these changes were much more dramatic in the obese rats suggesting that they need more "signal" for the feeling of satiety at the VMH-PVN level (47). Glucoprivic feeding or satiety are induced in normal rats by intravenous infusions of insulin or insulin + glucose respectively, while the Zucker rat are resistant to these treatments. The monoaminergic changes brought about by these infusions (insulin and insulin + glucose) were similar in obese and normal rats (namely a decrease in serotonin and dopamine and an increase in 5-HIAA and DOPAC). But the occurrence of meals, in the obese, showed monoaminergic changes resembling those related to spontaneous feeding. At the beginning of meals presented for the first time, VMH-PVN immunoreactive insulin increased earlier and with a smaller magnitude in the obese. When the rats were accustomed to scheduled meals, a similar anticipatory increase in immunoreactive insulin was found in both obese and lean rats. These results suggest that brain insulin is not just a satiety signal. In addition, in response to an intravenous insulin infusion, immunoreactive insulin in the brain increased twice as much in obese rats despite lower basal levels. This indicates that the larger response of the obese Zucker rat, known to be insulin resistant, reflects the inefficiency of the peptide in reducing feeding and body weight in the Zucker rats. In other words, there is a mismatch between the changes in the insulin levels in the brain and the corresponding changes in the monoamines in VMH and PVN during fasting and after feeding. Thus, there is an increase in the production of insulin from the pancreatic beta cells and simultaneously there is also an increase in the levels of insulin in the brain. These changes in the plasma and brain levels of insulin stimulate the production and release of monoamines in VMH and PVN. Once the feeding is over and the plasma glucose levels achieve normal range (compared to the fasting levels) the plasma and brain insulin levels also fall and this would trigger the return of monoamines to normal. Though both in the lean and obese similar changes were found in the levels of insulin and monoamines, the responses seen in the obese were comparatively defective, emphasizing the fact that obese rats and probably diabetic animals (and humans) have what is called as "Reward deficiency syndrome". Thus, the changes in the monoamines noted in the hypothalamus are insufficient to trigger the feeling of satiety and hence, they continue to consume food despite the fact that plasma insulin and glucose levels are normal or even higher. In other words, the responses of the monoamines in the hypothalamus are inadequate both to the feeding and given plasma and brain insulin levels. This resistance to insulin is reflected in inappropriate response

seen in the monoamines. Hence, these obese animals (and humans) need supraphysioloical increases in plasma glucose, insulin and monoamines to signal the satiety feeling. In addition, the mismatch between plasma and brain insulin and hypothalamic monoamines suggests that the cross-talk between these two systems is defective in the obese.

Thus, there are some very specific changes in the monoaminergic innervations of the central nervous system both in obesity and type 2 diabetes mellitus (48). The monoaminergic innervations of the central nervous system are characterized by long and short projecting neurons. It was reported that the long serotoninergic axons innervating the spinal cord and the cerebral cortex were unaffected in diabetic animals and that the noradrenergic innervations of the cortex was normal as well. The serotonin content was higher (almost twice as high) in the hypothalamus with no change in 5-HIAA levels, suggesting supernumerary innervation that is accompanied by a reduced release. In pons medulla oblongata, serotonin and dopamine and the metabolites 5-HIAA and DOPAC were significantly reduced, whereas noradrenaline was markedly increased. In the hippocampus, there was a reduction of serotonin content. The distal projections of serotonin were normal accompanied by hyperinnervation of the hypothalamus but the shorter collaterals were lost in the pons medulla oblongata. In the hypothalamus and in the striatum of diabetic rats, there were significant higher levels of substance P and met-enkephalin, respectively. The abundance of proenkephalin A mRNA is also increased in the striatum. Conversely, in the lumbar cord of diabetic animals, the levels of substance P and met-enkephalin were significantly reduced. These alterations suggest that there is retrograde degeneration of the peripheral sensory input. It has been suggested that these alterations are due to lack/deficiency of insulin, which could have triggered these monoaminergic alterations in the diabetic brain. These changes in the monoaminergic nerves coupled with the observation that there are some very specific interactions between insulin and hypothalamic monoamines indicates that insulin secretion, action and insulin responses to hunger and food function as a closely knit unit. In addition, similar if not identical changes were reported in diabetic neuronal dystrophy of the gut: noradrenergic sympathetic axons hyperinnervate the duodenum of diabetic rats, whereas noradrenaline levels were significantly reduced in the jejunum. Enteric neurotransmitter dopamine levels were elevated in the duodenum. Substance P and met-enkephalin content were remarkably reduced throughout the small intestine, whereas vasoactive intestinal polypeptide levels (VIP) were significantly increased in the duodenum, whereas the intrinsic serotoninergic innervation of the gut was not affected (49). These results indicate that the changes of gut innervations observed in experimental diabetes are consistent with increased content and also likely with hyperinnervation by the neuronal systems involved in smooth muscle relaxation and decreased content and with denervation by those systems with smooth muscle contraction properties that would explain the gastrointestinal dysfunctions seen in diabetes mellitus.

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It is possible that subtle changes in the sympathetic and parasympathetic nervous systems and monoamines could alter intestinal function in such a way that it would affect the secretion of the digestive juices, process of digestion and absorption of the nutrients that could ultimately influence the initiation, development and maintenance of obesity and its consequences such as type 2 diabetes mellitus and the metabolic syndrome. Since, intestinal microbiota plays a significant role in obesity (50), the gut bacterial quality and quantity depends on the diet ingested, intestinal epithelial cells and gut associated lymphocytes and their responses to the intestinal microbiota. It is possible that gut associated lymphocytes could secrete soluble factors (this may include cytokines, monoamines and other known and unknown factors) that can alter the growth pattern of intestinal microbiota. It is likely that the production of such soluble factors depends on the gut innervations. Since insulin has the ability to alter the production of dopamine, serotonin and noradrenaline and other monoamines and peptides, it is reasonable to propose that even in obesity and insulin resistance there could occur changes in the sympathetic and parasympathetic innervations of the gut and their respective secretory products.

There is evidence to suggest that central neuronal activity has a regulatory role in cytochrome c oxidase function. When central neural activity was measured relative to cytochrome oxidase activity in VMN (that regulates thermogenesis regulation), parvocellular paraventricular nucleus (PVN, that regulates feeding), and the magnocellular PVN (that controls the secretion of vasopressin and oxytocin) it was noted that cytochrome oxidase activity was significantly lower in the VMN and parvocellular PVN but not in the magnocellular PVN in the obese (fa/fa) rats compared to the lean rat. Determination of corresponding differences in levels or release of hypothalamic monoamines at rest and after exposure to 2 hours of 9^0C, it was observed that the concentrations of: 5-hydroxyindoleacetic acid (5HIAA; metabolite of serotonin, 5HT) in the VMN; 3-methoxy-4-hydroxyphenylglycol (MHPG; metabolite of norepinephrine, NE) and NE + MHPG (index of total NE) in the preoptic area; and 3,4-dihydroxyphenylacetic acid (DOPAC; metabolite of dopamine, DA) in the PVN were lower in the obese compared to the lean. Exposure to cold resulted in elevation in the VMN of concentrations of MHPG and MHPG + NE in both lean and obese rats; elevated concentrations of 5HT, 5HIAA, and 5HT + 5HIAA in obese rats, with no significant changes in these variables in lean animals; decreased ratio of 5HIAA/5HT in obese rats and increased ratio in leans. In the preoptic region, cold exposure led to increased concentrations of MHPG, NE + MHPG, 5HT, and 5HT + 5HIAA in obese but not lean rats. In the PVN, 5HT concentrations were increased in cold-exposed obese but not lean rats. These results clearly suggest that the neuronal activity in the obese are different from that of lean both at rest and during cold exposure and indicates that hypothalamic monoamines play a significant role in the regulation of thermogenesis on exposure to cold and temperatures (51). Since energy expenditure especially, non-basal energy expenditure is a permissible factor for obesity, these results imply that by influencing the monoamine oxidase activity hypothalamic monoamines play significant role in the pathobiology of obesity and its association with insulin resistance. Since insulin has a modulatory influence on the secretion and action of hypothalamic monoamines and vice versa, and as the concentrations of hypothalamic monoamines are closely related to the activity of cytochrome oxidase activity, it is reasonable to propose that there is a close interaction among hypothalamic monoamines, insulin secretion and action and insulin resistance seen in obesity. Thus, it is likely that insulin resistance could very well be a disease of the brain.

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Abbreviations: CHD: coronary heart disease, VMH: ventromedial hypothalamus, CNS: central nervous system, HPA: hypothalamo-pituitary-adrenal, oCRF: ovine corticotropin-releasing factor, OGTTs: oral glucose tolerance tests, GLUT-4: glucose transporter 4, NPY: neuropeptide Y, MC: melanocortin, POMC: pro-opiomelanocortin, ACTH: adrenocorticotrophin hormone, MSH: melanocortin stimulating hormone, MC3-R: melanocortin 3 receptor, MC4-R: melanocortin 4 receptor, LSNA: lumbar sympathetic nerve activity, MAP: mean arterial pressure, BAT: brown adipose tissue, i3vt: third intracerebroventricular, ICV: intracerebroventricularly, LCGU: local cerebral glucose utilization, ARC: arcuate

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hypothalamic nucleus, DN: dorsomedial hypothalamic nucleus, VMN: ventromedial hypothalamic nucleus, Irs: insulin receptors, NIRKO mice: neuron-specific disruption of the IR gene mice, FIRKO mice: mice with fat-specific disruption of the insulin receptor gene, SHC: homology adaptor protein, GRB2: growth factor receptor binding protein-2, SHP2: SH protein tyrosine phosphatase-2, IR-beta : insulin receptor beta subunits, IRS-1: insulin receptor substrate 1, IRS-2: insulin receptor substrate 2, NE: norepinephrine, DA: dopamine, MHPG: 3-Methoxy-4-hydroxy-phenylglycol, 5-HT: 5-hydroxytryptamine, DOPAC: 3, 4-dihydroxyphenylacetic acid, 5-HIAA: 5-hydroxy-3-indole acetic acid, DHPG: dihydroxyphenylethylene glycol, CN: caudate nucleus, PM: pons-medulla, PVN: paraventricular nucleus, VIP: vasoactive intestinal polypeptide

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