Dopamine D2 receptors in the pathophysiology of insulin resistance

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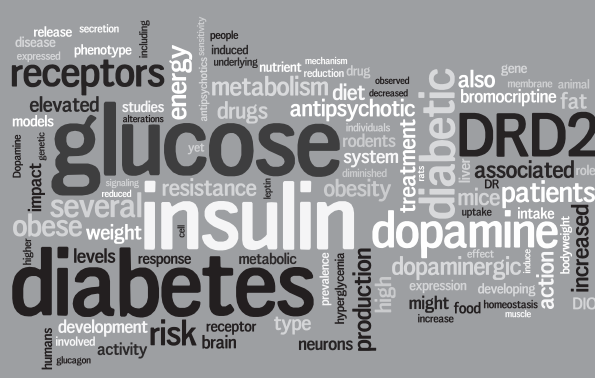
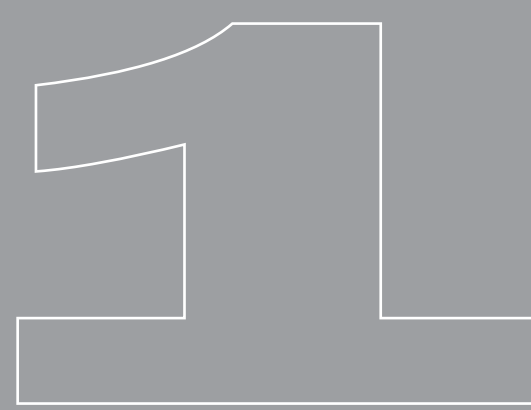


General Introduction



Diabetes

Characteristics

The prevalence of Diabetes Mellitus Type 2, also known as non-insulin-dependent diabetes or adult-onset diabetes, is rising alarmingly. In 1985 approximately 30 million people worldwide suffered from diabetes. In 2007 this number had escalated to 246 million and by 2030 it is expected that ~ 438 million people (7.8% of the adult population) will be affected by diabetes[1]. At present, especially the developed world is coping with the diabetes epidemic, the prevalence in the US being 12.3% and in the Netherlands 7.7%, yet the developing countries are rapidly catching up[1]. It is estimated that, in the developing countries, the prevalence of diabetes will more than double in the years 2000-2030, compared to an increase of merely 50% in the western world[2].

Diabetes is a major cause of mortality. According to the WHO, diabetes has reached the top 10 of death causes in middle and high income countries[3]. It is predicted that in 2010 almost 4 million deaths will be attributed to diabetes, which represents 6.8% of global all-cause mortality[4]. The mortality risk for individuals with diabetes is 2.3 times higher than the risk for people with normal glucose homeostasis[5]. Cardiovascular disease, which is a frequently encountered complication of diabetes, is the main reason for the elevated mortality risk. Compared to the general population, diabetic people younger than 45 years are 10 times more likely to display cardiovascular disease, ranging from relatively mild (hypertension and atherosclerosis) to severe (stroke and myocardial infarction)[6]. Approximately 16% of diabetic patients suffer from severe cardiovascular incidents leading to hospital admission; this risk is ~ 2.3 fold higher than for non-diabetic subjects[7,8]. In addition, the risk of mortality due to cardiovascular disease is 2.6 times higher in diabetic patients[5].

Long term diabetes and poor glycemic control also lead to several other seriously disabling disorders. Diabetic nephropathy e.g. is one of the major causes of end-stage renal failure in the Western world[9]. Approximately 1.2% of diabetic patients develop renal failure, which represents a ~ 4 times higher risk than observed for people without diabetes[7,8]. Also, diabetes is the leading cause of new cases of blindness among adults aged 20-74 years[10]. The risk of developing any ophthalmologic complication, including cataract, glaucoma and diabetic retinopathy, is ~ 3 times elevated in diabetic versus nondiabetic individuals[8]. And, ~ 50% of diabetic patients develop neuropathy, which might manifest as sensory loss, muscle weakness, pain and/or erectile dysfunction[10,11].

Aetiology

Type 2 diabetes originates from a complex interplay between genetic and environmental factors. The contribution of a genetic component in the development of diabetes is undeniable, given the observation of an

extremely high diabetes prevalence among certain population groups like the Pima Indians[12,13]. Likewise, the high concordance rate of diabetes among both monozygotic and dizygotic twins suggests a genetic component to the disease[14,15]. And first-degree relatives from diabetic patients display several defects in energy and nutrient metabolism[16,17].

Some forms of type 2 diabetes, such as the different types of MODY (Maturity-Onset Diabetes of the Young), are of monogenic origin, meaning that one gene is responsible for the disease[18]. These forms of diabetes are characterized by a single gene mutation, an autosomal dominant inheritance pattern and an early onset of the disease. These cases however, represent only about 1-5% of all type 2 diabetes cases[18]. The majority of type 2 diabetes is of polygenic origin, meaning that several susceptibility genes additively increase the risk of disease onset. The contribution of single susceptibility genes to the diabetes risk is generally small; with odds ratios between 1.10 and 1.30. However, if several susceptibility loci are present, the risk of developing diabetes may increase substantially, as was shown for a Japanese population in which the risk of developing diabetes increased ~ 3.7 fold in the presence of a combination of 7 specific susceptibility loci[19]. Association studies in large population cohorts revealed several susceptibility genes, including PPARγ, TCF7L2, KCNJ11, CDKAL1, CDKN2A/CDKN2B, IGF2BP2, SLC30A8 and HHEX[19-21].

The contribution of the genetic predisposition is believed to remain stable throughout time; therefore it can not explain the recent rapid increase in diabetes incidence. Rather, this has been triggered by advances in health care and lifestyle changes. The prevalence of obesity, which is a major risk factor for diabetes development, has increased considerably the last decennia. In the US, the prevalence of adult obesity rose from 13.4% in 1960 to 30.9% in 2000[22] and the number of overweight children aged 6-11 and 12-19 increased from 4% and 6% in 1971 to 15.3% and 15.5% respectively in 2000[23]. The rise in diabetes incidence may greatly be accounted for by the recent rise in number of obese subjects. An objective measure to describe obesity is the body mass index (BMI), which is calculated as weight (in kilogram) divided by the square of the height (in meters); a BMI of < 18.5 represents underweight, 18.5-25 normal weight, 25-30 overweight, 30-35 obesity and > 35 morbid obesity. The lifetime risk for developing diabetes rises dramatically with increasing BMI. For an 18-year old person with normal weight, the risk of developing diabetes was calculated to be ~ 18.5%, if this person was morbidly obese though, the risk would increase to ~ 72%[24]. The predisposition of obesity to turn into diabetes is also reflected by the observation that in the US ~ 55% of type 2 diabetic patients is obese[25].

The change from an active to a sedentary lifestyle, promoted by the industrialization, the availability of easy transportation and the introduction of computers, television and video games, also independently adds to the elevated diabetes prevalence. A prospective cohort study in the US showed that with

every additional 2 hours of TV watching daily, the risk of diabetes increases with 14% and for every 2 hours/day increase in sitting at work, the risk for diabetes rises with 7%[26]. On the opposite, the impact of physical activity on reducing the risk of diabetes development has also firmly been established[27-31]. It is calculated that each 500 kcal increment in energy expenditure per week leads a 6% decrease in diabetes risk[27]. Even in people with impaired glucose tolerance, representing a pre-diabetes stage, physical activity is beneficial and reduces the risk of overt diabetes with 46%[32]. Clinical trials in patients with overt diabetes also indicate that physical activity, without weight loss, is able to improve the diabetic phenotype[33-35].

Also, the altered dietary pattern participates in the increased incidence of diabetes. With the introduction of highly palatable, energy-dense, food, total caloric intake increased and the dietary preferences shifted away from the traditionally "healthy" diet, including vegetables, fruits, low-fat dairy products and whole grain products, towards the "western type" diet, comprised of red and/or processed meat, high fat diary products, refined grain products, fried products and sweet beverages. Analyses of the health risk/benefit of both types of diets indicated that consumption of the "western type" diet is associated with a 28-60% higher risk of developing diabetes, while the "healthy" diet is associated with a modestly protective effect of 11-27%[36-39].

Finally, improved health care, which dramatically increased life expectancy the past decades, accounts for part of the elevated diabetes incidence. Aging is associated with an increased prevalence of diabetes; in the US, in the period of 2005-2006, the prevalence of previously diagnosed diabetes was 2.1% in the age group 20-39, 7.9% in the age group 40-59 and 17.6% in the age group 60-74[40]. Therefore increased longevity will greatly enlarge the number of diabetes patients. It is still a matter of debate whether the increased diabetes risk for elderly people is the result of aging per se or the result of age-related alterations in lifestyle and body composition. Unhealthy diets, decreased physical activity, increased adiposity and an altered fat distribution are all phenomena associated with aging and independent risk-factors for the development of diabetes. Accordingly, several studies showed an age-related deterioration of insulin action, yet in some, the reported differences between young and old individuals were diminished, or even completely lost, when corrected for age-related risk factors[41-45].

Glucose homeostasis

Physiology

Plasma glucose levels are maintained within a narrow range of 4-7 mmol/l. If glucose levels fall below the threshold of 3 mmol/l, energy supply to the brain becomes inadequate. The brain is unable to use substrates other than

glucose for energy and is only equipped with glycogen stores sufficient for a few minutes. Therefore, hypoglycemia rapidly leads to functional brain failure, seizures and coma. If the hypoglycemia is severe and prolonged it might even lead to brain death[46]. Conversely, elevated glucose levels can damage organs leading to macrovascular disease, nephropathy, retinopathy and neuropathy[47].

Insulin and glucagon are the key hormones regulating glucose homeostasis. Insulin is secreted by pancreatic β-cells in response to a physiological rise in glucose levels, e.g. after a meal. The net effect of insulin is to reduce the elevated glucose levels by promoting glucose uptake and simultaneously inhibiting glucose production. The liver is the main site responsible for the production of glucose. It can either convert stored glycogen into glucose or synthesize glucose de novo from non-carbohydrate substrates including lactate and amino acids. In insulin sensitive tissues like muscle, adipose tissue and also liver, glucose can be taken up and subsequently converted into glycerol for storage or oxidized to supply energy.

Glucagon is secreted by pancreatic α-cells in response to a reduction in blood glucose concentrations, e.g. during fasting. Opposing the action of insulin, glucagon increases glucose levels. It promotes the production of glucose by the liver, leading to an induction in both the conversion from glycogen to glucose and de novo glucose synthesis. Concomitantly glucagon inhibits the synthesis of glycogen and the oxidation of glucose in the liver[48]. During conditions of hyperglycemia glucagon production is suppressed by the combined action of the elevated glucose levels and the concomitantly raised insulin levels[49]. Other physiological regulators of glucose homeostasis include glucose, which can regulate its own disposal and release, catecholamines, cortisol and growth hormone.

Pathophysiology

Hyperglycemia is an important hallmark of diabetes and is the direct corollary of dysregulation of insulin and glucagon action. Impaired insulin action involves both insulin resistance, a reduced ability of tissues to respond to insulin, and defects in insulin secretion. In the early development of insulin resistance the diminished efficacy of the hormone is overcome by elevated insulin production by pancreatic β-cells; hyperinsulinemia therefore is a marker for diabetes development. Eventually, β-cells are no longer able to produce sufficient amounts of insulin to compensate for the resistance. Consequently, the biological function of insulin is undermined and hyperglycemia becomes manifest.

Insulin resistance can be demonstrated as a reduction in whole body glucose uptake and diminished suppression of glucose production during conditions of hyperinsulinemia. Several organ-specific mechanisms are thought to underlie the impaired insulin sensitivity.

Together, muscle, adipose tissue and liver are responsible for glucose disposal in response to insulin. As muscle tissue is the major contributor, insulin resistance of this tissue will greatly impair the ability of the body to remove glucose from the circulation. In response to insulin, GLUT4, the insulin-responsive transporter mediating the diffusion of glucose across the cell membrane, is translocated to the cell membrane. Once glucose has entered the cell, it is rapidly phosphorylated in order to maintain a concentration gradient for glucose across the cell membrane. In muscle cells from diabetic patients both the insulin induced transport of glucose across the cell membrane and the subsequent phosphorylation of intracellular glucose are diminished[50-52]. Several alterations in the intracellular signaling pathways downstream of the insulin receptor have already been described[53-55]. Together these might lead to a diminished recruitment of GLUT4 from intracellular storage vesicles to the cell membrane giving rise to the reduced glucose uptake[51].

Also in adipose tissue from diabetic individuals several defects have been noted. Diminished binding of insulin to its receptor in combination with a reduced receptor kinase activity greatly impairs the insulin action on adipocytes[56,57]. Concomitantly, both the basal expression of GLUT4 transporters on the cell membrane and the insulin stimulated translocation of GLUT4 to the surface is decreased in adipocytes from diabetic patients[57,58]. These latter observations might be ascribed to an enhanced turnover of glucose transporters and/or a diminished transporter gene expression[57,58].

Glucose uptake by the liver is mainly relevant after a meal, when both plasma glucose and insulin concentrations are elevated[59]. In diabetic patients, the capacity of the liver to extract glucose from the circulation under these postprandial conditions is compromised[60,61] as well as its ability to synthesize glycerol[62]. In contrast to muscle, where glucose transport across the plasma membrane is the rate-limiting step for glucose uptake, in liver, phosphorylation of glucose, by the enzyme glucokinase, is rate-limiting. Therefore, decreased activity of this enzyme, found in diabetic subjects[63,64], might be responsible for the reduced glucose uptake.

Concomitantly, the role of the liver as main producer of glucose is affected. Total, as well as directly measured hepatic, glucose production is higher in diabetic patients, both during basal, fasting, conditions[60,65-67] and hyperinsulinemic, fed, conditions[67,68]. The direct corollary is fasting and postprandial hyperglycemia. The contribution of increased gluconeogenesis to the elevated glucose production in diabetic subjects has firmly been established, but the contribution of glycogenolysis is still a matter of debate[65,66,68]. An increased ratio of the activity of the enzyme glucose-6-phosphatase to glucokinase, measured in diabetic patients, might contribute to the elevated glucose production[64].

Another contributory factor to the pathophysiology of hyperglycemia is an elevation in glucagon levels. In type 2 diabetic patients the postprandial suppression of glucagon production is impaired[69,70] leading to hyperglucagonemia and, regarding the nutritional status, an inappropriate stimulation of glucose production by the liver[71]. Possibly, resistance of pancreatic α-cells to the inhibitory action of insulin underlies this phenomenon.

Defective insulin secretion, which is, in addition to insulin resistance, an obligatory step in the development of type 2 diabetes, is the result of both a decrease in β-cell mass and β-cell malfunction. The reduced β-cell mass observed in type 2 diabetic patients is presumably the net effect an accelerated apoptosis rate in combination with normal β-cell replication and neogenesis[72,73]. Physiological signs of insulin secretion defects include an absence of the first phase insulin response[74,75], alterations in the pulsatility of insulin secretion[76,77] and an increased proinsulin to insulin ratio[78,79]. Intracellular defects underlying this β-cell malfunction include a reduction in the expression of glucose transporters GLUT1 and 2, impaired intracellular glucose processing[75] and a loss of insulin gene expression[80]. Damage and death of β-cells may be the consequence of hyperglycemia per se, as stated by the glucotoxicity theory. Accordingly, it was shown that prolonged hyperglycemia, either in combination with high circulating FFA levels or alone, promotes apoptosis and alterations in key components of cellular functioning through long-term increases in cellular Ca^{2+} concentrations[81] and oxidative stress[80]. Alternatively, or additionally, hyperglycemia may induce defects indirectly by promoting hypersecretion of insulin, leading to β-cell exhaustion[82].

Diabetic rodent models

Currently, several different rodent models have been established for diabetes research. Although most of these animal models fail to develop overt hyperglycemia and diabetes related complications, they do develop a diabetes-like phenotype characterized by obesity and insulin resistance. Some of these rodents models are genetic models; the result of single gene alterations. Three frequently used genetic models for diabetes research are the obese Zucker rat, the ob/ob mouse and the db/db mouse, all of which are characterized by mutations in genes involved in leptin signaling. The hormone leptin, predominantly synthesized by adipose tissue, serves as a regulator of long-term energy balance. Leptin is secreted in proportion to the amount of body fat and is therefore able to convey information about peripheral energy reserves. The long isoform of the leptin receptor is expressed in several regions of the brain, including the hypothalamus, and transmits the 'anti-obesity' action of leptin on food intake and energy expenditure[83]. In obese Zucker rats and db/db mice, respectively, a mutation in, and a deletion of, the leptin receptor were

found[84,85], whereas in ob/ob mice a mutation in the leptin gene was discovered[86]. Resistance to the physiological action of leptin, as well as the absence of leptin, leads to the development of a diabetogenic phenotype, including hyperphagia, reduced energy expenditure, obesity and insulin resistance[87-90].

Another genetic rodent model is the OLETF rat. These rats, presenting several of the characteristic features of diabetes, are naturally occurring CCK-1 receptor knockouts[91,92]. Cholecystokinin (CCK) is a peptide released from the gastrointestinal tract in response to food intake. CCK action is mediated by 2 distinct receptors which are both expressed in the periphery as well as in the brain. CCK regulates digestive function and promotes satiety. The CCK-1 receptor is responsible for the latter function[93].

Although the animal models described above, displaying spontaneous mutations in essential metabolic pathways, have provided us with important information concerning energy and nutrient balance, they only represent a small portion of the heterogeneous human diabetes population, since only a small percentage of diabetes cases are the result of single gene mutations. Diet induced obese (DIO) rodents better reflect the complex physiological alterations underlying the disease in the majority of obese type 2 diabetic patients. Several wild type (wt) rodents, such as the C57BL/6J mice, develop a diabetic phenotype after being fed a high fat diet for several weeks[94,95]. This DIO animal model is often used in diabetes research, yet, it is still a heterogeneous group; on average, DIO rodents develop a diabetic phenotype, but, there are large differences in the adaptation of individual animals to high fat feeding. It was shown that, after being maintained on a high fat diet for 9 months, 45% of a group of C57Bl6 mice became obese and diabetic, 12% remained lean and non diabetic, 12% was lean and diabetic and 30% showed an intermediate phenotype[96]. The insulin resistance phenotype of lean diabetic mice resembled more the phenotype of lean non-diabetic mice than of obese diabetic mice, so, simplified, the C57Bl6 mice could be divided into a diet induced obese (DIO), a lean diet resistant (DR) and an intermediate group. For experimental purposes wt rodents, while still maintained on a chow diet, can be divided into DIO and DR groups according to the amount of norepinephrine they excrete[97]. Alternatively, wt rodents can be classified according to their weight gain following several weeks of high fat feeding; the rodents with the highest weight gain are designated DIO and those with the least weight gain, DR[98].

Considering the heterogeneous response of humans towards the diabetogenic western type diets, we believe the DIO/DR rodent model accurately represents the human situation and is therefore best suited for analyzing the complex metabolic alterations associated with the development of obesity and diabetes.

Dopaminergic system

Physiology

Dopamine is the predominant catecholamine neurotransmitter in the mammalian central nervous system. It is synthesized in dopamine neurons and stored in synaptic vesicles until release. Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the conversion of tyrosine into dopamine. Activation of dopamine neurons promotes fusion of the synaptic vesicles with the neuronal membrane, and dopamine is secreted. Upon release, dopamine binds to its receptor, located either on pre- or postsynaptic neurons, and initiates an intracellular signaling cascade. Dopamine transporters (DAT) take up dopamine from the extracellular fluid, thereby rapidly limiting the activity of secreted dopamine. Back in the neuron, dopamine is either transported into synaptic vesicles by a vesicular monoamine transporter (VMAT2) to be re-used or it is metabolized by monoamine oxidase (MAO) or catechol-O-methyltransferase (COMT)[99,100].

Dopamine neurons are present in distinct areas of the brain, giving rise to three main dopaminergic pathways. The nigrostriatal pathway contains dopamine neurons originating in the substantia nigra and projecting to the dorsal striatum. Dopaminergic signaling in this pathway controls locomotor activity. The mesocorticolimbic pathway consists of dopamine neurons projecting from the ventral tegmental area to the ventral striatum, the limbic system and the cortex and is involved in emotion, cognition, motivation and reward. The dopamine neurons comprising the tuberoinfundibular pathway originate in the hypothalamus and project to the pituitary where they control hormone secretion and cell survival[101,102].

In addition to their role in the tuberoinfundibular pathway, dopamine neurons located in the hypothalamus control ingestive behavior. These neurons receive signals concerning energy homeostasis and nutrient availability from the periphery through afferent nerves, circulating hormones, nutrients and small peptide mediators. They integrate the information and relay it to the classical food intake-related neurons including NPY/ AGRP producing neurons (stimulators of food intake) and POMC producing neurons (inhibitors of food intake) to direct energy intake[103].

Dopamine receptors

Dopamine action is mediated by 5 distinct receptors which are categorized into 2 receptor families based on sequence homology and pharmacological characteristics. The D1-like family consists of the dopamine receptors D1 (DRD1) and D5. Activation of these receptors leads to stimulation of adenylyl cyclase and the subsequent production of cyclic AMP. Activation of the D2-like family on the contrary, inhibits adenylyl cyclase activity and the concomitant

production of cyclic AMP. The receptors DRD2, DRD3 and DRD4 represent the D2-like family[101]. Apart from different functional characteristics, the dopamine receptors also differ in spatial expression patterns. DRD1 and DRD2 are the most widely expressed receptors; they are found in all brain areas receiving dopaminergic innervation. The other dopamine receptors display more restricted expression patterns[101,102]. Although dopamine D1 and D2 receptors are present in the same brain areas, they are only occasionally expressed on the same neurons[101].

Dopamine receptors belonging to the DRD2 family exist both as pre- and postsynaptic receptors. Presynaptic receptors, or autoreceptors, which are believed to be mainly DRD2 and DRD3, are part of a dopaminergic feedback mechanism regulating neuronal activity and neurotransmitter release. Accordingly, stimulation of autoreceptors can alter firing rate of the neuron, dopamine synthesis and secretion. Postsynaptic receptors, which can be either DRD2, DRD3 or DRD4, modulate the action of second order neurons in response to dopamine[100,101].

Peripheral dopaminergic system

Dopamine receptors are highly expressed in the central nervous system, yet they are also present in several peripheral tissues, orchestrating a variety of biological functions. In the cardiovascular system, dopamine receptors are involved in the regulation of blood pressure. In the heart, up till now, D1, D2 and D4 dopamine receptors have been described. The role of the individual receptors has not yet been defined, but overall, a low concentration of dopamine is associated with an increased cardiac output due to improved contractility of the heart[104-106]. All dopamine receptors are expressed in the systemic blood vessels where they control vascular resistance by regulating vasodilatation[107-109].

In the kidney, dopamine, in general, increases renal blood flow and the excretion of water and ions such as sodium and calcium. The participation of the individual dopamine receptors in this effect is complex and varies depending on several factors such as systemic water and sodium balance[109,110]. Dopamine receptors D1, D2, D4 and D5 are also present in the adrenal glands[111,112]. The D2-like receptors are known to control aldosterone production, but the function of the D1-like receptors hasn't been clarified yet[112-114].

All dopaminergic receptors are expressed in the gastrointestinal tract. The dopamine D2 receptor is involved in the inhibition of gastric acid production[115] and gastrointestinal motility[116,117]. The role of the other dopaminergic receptors remains unclear.

Furthermore, all dopamine receptors, except DRD1, are expressed on peripheral blood leukocytes[118,119]. The action of dopamine on immune cells has best been studied in lymphocytes. In these cells dopamine exerts a dual role;

activating resting lymphocytes and inhibiting activated ones[120,121].

Recently dopamine receptors were also discovered on pancreatic β-cells. The dopamine D2 receptor is clearly involved in the modulation of insulin secretion, but the role of the other receptors remains to be elucidated[122].

DRD2 and diabetes

DRD2 polymorphisms

Several lines of evidence link the dopaminergic system to obesity, insulin resistance and type 2 diabetes in humans and animal models. An important indication for a functional relationship between dopamine and metabolic disturbances came from epidemiological studies. Several groups have examined the association of DRD2 polymorphisms and energy and nutrient metabolism. Although in general the impact is small, there is an interaction between DRD2 variants and energy homeostasis. The polymorphism Ser311Cys, which impairs the DRD2 signal transduction pathway[123], is associated with a higher BMI and lower resting energy expenditure in Pima Indians[124,125]. The TaqIA1 allele, resulting in lower DRD2 binding[126], is associated with obesity[127,128]. And, a haplotype consisting of 2 SNP's located in intron 5 and exon 6 of the DRD2 gene is associated with obesity as well[129]. Recently, proof for a role of DRD2 in the regulation of glucose and insulin metabolism was provided by Guigas et al. who showed that, in humans, the rate of glucose stimulated insulin secretion is associated with a 4-SNP haplotype (including TaqIA1 SNP) of the DRD2 gene[130].

DRD2 neurotransmission

More evidence came from the analysis of the dopaminergic system in obese and diabetic animals and humans. The expression of DRD2 is reduced in specific brain areas of obese Zucker and OLETF rats compared to lean control rats[131-133]. This decreased DRD2 expression is also observed in the striatum of obese humans. Moreover, in these individuals the number of DRD2 binding sites is inversely related to the body mass index[134]. Additionally, basal dopamine levels are increased in the hypothalamus of obese diabetic rats and the dopamine release in response to food intake is exaggerated and longerlasting[135-138]. A higher dopamine concentration was also measured in post mortem brains of diabetic patients compared to controls[139]. The reduction in dopaminergic neurotransmission elicited by a decreased DRD2 expression is thought to induce a "reward deficiency syndrome", which might be compensated by elevated dopamine release and additionally, or alternatively, by "reward seeking behavior", such as increased food intake[140].

DRD2 antagonists

Another indication that dopamine D2 receptors might be involved in energy

and nutrient metabolism came from the clinical observation that the use of antipsychotic medication is associated with obesity, insulin resistance and diabetes. Although numerous different antipsychotic drugs are used in clinic, the common denominator of these drugs is their affinity for dopamine D2 receptors. In general, the newer, second-generation 'atypical' antipsychotics have a broader range of action and a slightly lower affinity for the D2 receptor compared to first-generation 'typical' antipsychotics, but they are still (at least partial) DRD2 antagonists. In fact, it has been suggested that the clinical efficacy of these drugs to alleviate psychotic symptoms depends on the interaction of the drugs with dopamine D2 receptors[141].

Most antipsychotic drugs induce some degree of weight gain, yet the atypical, second generation, drugs clozapine and olanzapine are associated with the most severe increase in body weight[142-144]; in a meta-analysis it was calculated that both drugs can induce weight gain of up to 4.5 kg in 10 weeks in schizophrenic patients[142]. Other antipsychotic drugs, such as the typical drug haloperidol, induce much less weight gain[142,144].

The use of antipsychotic drugs is also linked to the development of diabetes[143,145]. Again, treatment with clozapine or olanzapine is associated with the greatest risk of developing diabetes[145]. One study even showed that, in a health care center, 36.6% of patients on clozapine treatment were newly diagnosed with diabetes within 5 years after the start of treatment[146]. Although the metabolic side effects of antipsychotic drugs have been observed in schizophrenic patients and schizophrenia itself contributes to the increased risk of developing diabetes[147,148], it is generally accepted that antipsychotics can directly affect energy and nutrient metabolism. This is confirmed by studies in animal models and healthy humans.

As in schizophrenic patients, weight gain is consistently observed in healthy volunteers treated with the antipsychotics olanzapine and risperidone[149-153]. The impact of antipsychotic drugs on glucose metabolism in healthy individuals though, is less clear; some studies report a reduction in insulin sensitivity following drug treatment[149,151,152], whereas others fail to observe an effect on insulin sensitivity[150,153]. In rodents the ability of antipsychotics to induce weight gain seems to be gender specific; female rats are sensitive to the weight inducing effect of the drugs, whereas in most studies using male rodents, body weight is not affected, or even decreased, by drug treatment[154-158]. The impact of antipsychotics on glucose metabolism, however, is consistent in animals. Both chronic and acute antipsychotic drug treatment is highly associated with the development of glucose intolerance and insulin resistance[156,159-164].

Alterations in several pathways might underlie these antipsychotic induced metabolic abnormalities. In most animal experiments, antipsychotics induce a defect in insulin stimulated glucose uptake during hyperinsulinemia[161-164]. Accordingly, it was observed that several antipsychotic drugs reduce glucose

uptake in neuronal cells[165]. The inability of tissues to appropriately respond to insulin stimulation might depend on an antipsychotic induced defect in insulin signaling, as is described in muscle cells after incubation with olanzapine[166].

In addition, an abnormally high endogenous glucose production during hyperinsulinemia is found in several animal models on antipsychotic drug treatment[159,161,164]. The underlying mechanism might be the inability of the liver to respond to the inhibitory action of insulin and/or the ability of antipsychotic drugs to acutely stimulate the endogenous glucose production, as is shown in rats[160].

A defect in insulin release might further add up to the metabolic alterations induced by antipsychotics. Several studies have reported an antipsychotic drug induced reduction in insulin response during hyperglycemia[162,163]. Accordingly, it was found that antipsychotic drugs can directly affect insulin release from isolated pancreatic islets[167-169].

DRD2 agonists

Considering the impact of the dopaminergic system on energy and nutrient metabolism, several groups have examined the efficacy of DRD2 agonists in ameliorating the adverse metabolic conditions associated with diabetes. The best studied DRD2 agonist in relation to obesity and diabetes is bromocriptine, clinically used in the treatment of Parkinson's disease and hyperprolactinemia. In humans, several trials have been performed with this DRD2 agonist. The most consistent impact of such treatment in obese individuals is normalization of elevated plasma glucose levels[170-173]. In addition, in several studies, bromocriptine treatment diminished basal plasma insulin levels in obese individuals[170,173]. The impact of bromocriptine on body weight though, is inconsistent among studies; in some the body weight and fat percentage of subjects decreased upon treatment[174], while in others body weight remained stable throughout the experiment[171-173]. The impact of bromocriptine on glucose metabolism is more consistent; it improves glucose tolerance and insulin sensitivity in obese people[172,174].

In most animal studies bromocriptine was given in combination with the DRD1 agonist SKF38393, as this latter drug enhances the efficacy of bromocriptine[175,176]. Unlike in humans, treatment of obese diabetic animal models with the combination of bromocriptine and SKF38393 consistently decreases food intake, fat mass and overall body weight[175,177-180]. Surprisingly, the decrease in food intake was only moderately involved in body weight reduction, as pair feeding was only able to partly reproduce this effect[179,180]. The impact of enhanced DRD2 stimulation on food intake and body weight has also been confirmed with quinpirole, another DRD2 agonistic drug[176]. Furthermore, like in humans, bromocriptine/SKF38393 treatment normalizes elevated plasma glucose and insulin levels in obese diabetic animals[175,177-180].

The underlying mechanism(s) for this improvement is not yet fully elucidated, but bromocriptine/SKF38393 treatment reduces the activity of 2 key enzymes involved in hepatic gluconeogenesis in obese insulin resistant mice[179] and glucose production is diminished in bromocriptine treated hamsters[181]. Also, bromocriptine improves glucose tolerance and insulin sensitivity[177,182]. This might be mediated by a restoration of the aberrant β-cell function by bromocriptine/SKF38393, resulting in a reduction of the elevated basal insulin release, an increase in insulin content and an improved glucose-stimulated insulin release[178,183,184].

Injection of bromocriptine directly into the brain of diabetic hamsters, in a concentration that does not have an effect when administered systemically, also diminishes body weight and improves glucose tolerance and insulin sensitivity[185]; suggesting that (part of) the observed effects of DRD2 stimulation on metabolism are mediated by dopamine receptors in the brain.

Outline of this thesis

The dopaminergic system in general and the dopamine receptor D2 specifically are functionally linked to diabetes-associated metabolic derangements. Genetic variations in the DRD2 gene are associated with altered energy and nutrient homeostasis. Inhibition of DRD2 promotes a diabetes-like phenotype, while activation of DRD2 restores a normal metabolic profile. Also several components of dopaminergic signaling are modified in obese and diabetic humans and animals. Despite the established interaction between DRD2 and disturbances in the energy and nutrient homeostasis, several questions regarding the exact role of DRD2 in the aetiology of diabetes and the mechanism underlying the metabolic corollary of DRD2 transmission modulation remain unanswered. The research described in this thesis is conducted in order to unravel the characteristics of the interplay between the DRD2 and glucose metabolism as well as to understand the underlying mechanism(s).

The aim of chapter 2 was to determine the role of the dopaminergic system in the aetiology of high fat diet induced obesity and insulin resistance. Therefore, glucose metabolism and several indicators of dopaminergic neurotransmission were evaluated after 4 weeks of high fat feeding in wt mice and compared to mice maintained on a low fat diet.

Calorie restriction is the most effective way to extend lifespan and reduce morbidity. As such, it also improves insulin sensitivity and delays the age-related loss of DRD2 expression in the brain. Considering this, together with the role of the dopaminergic system in glucose metabolism, it can be suggested that the dopaminergic system is involved in the beneficial impact of calorie restriction on insulin action. This hypothesis was addressed in chapter 3. Wt mice were maintained on a high fat diet, either with unlimited or restricted

access, for 12 weeks. During the entire experiment half of the calorie restricted mice also received continuous haloperidol treatment. After the treatment period glucose metabolism was evaluated and the hypothalamic DRD2 binding was determined.

In general, high fat feeding induces obesity, insulin resistance and a type 2 diabetic phenotype in rodents, but there is a large diversity in response within single strains of rodents. Based on weight gain, the phenotype of rodents on a high fat diet can be characterized as diet induced obese (DIO), intermediate or diet resistant (DR). DIO and DR rodents differ in several components of the dopaminergic system, even before the onset of obesity. This led to the suggestion that variation in dopaminergic neurotransmission participates in the development of the divergent DIO and DR phenotypes. Therefore, in chapter 4 we maintained wt mice on a high fat diet for 10 weeks to classify them as DIO and DR. Subsequently we treated DIO and DR mice with, respectively, the DRD2 agonist bromocriptine and the DRD2 antagonist haloperidol and performed indirect calorimetric measurements and characterized glucose metabolism. Placebo treated DIO and DR mice served as controls.

Antipsychotic drugs are associated with the development of insulin resistance and dyslipidemia. It is, however, still unclear if these drugs directly modify glucose and lipid metabolism or if they promote weight gain which may lead to the disturbed metabolic profile. Therefore, in chapter 5, the short-term impact of the typical antipsychotic drug haloperidol and the atypical drug olanzapine were studied in order to unravel the mechanism underlying the deregulation of nutrient metabolism. The carbohydrate and lipid metabolism of healthy men was evaluated before and after 8 days of antipsychotic drug treatment.

The DRD2 agonistic drug bromocriptine is highly effective in improving glucose metabolism and β-cell function, yet, the underlying mechanism remains unclear. In chapter 6 we studied the acute impact of bromocriptine on insulin secretion and action in wt mice and the impact on intracellular signaling in INS-1E cells.

In chapter 7 the results obtained with these studies and their implications are discussed.

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