Universiteit
Leiden
The Netherlands

Dopamine D2 receptors in the pathophysiology of insulin resistance

Leeuw van Weenen, J.E. de

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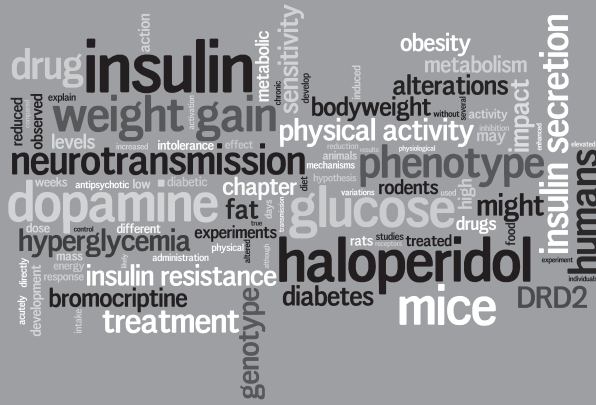

General Discussion

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The dopaminergic system controls a multitude of physiological functions, ranging from motor activity to hormone secretion and feelings of reward. Previously, it has also been implicated in glucose and insulin metabolism. Disruption of the glucose and insulin metabolism leads to insulin resistance and diabetes mellitus type 2. During the initial stages of diabetes development, insulin resistance will be compensated by an elevated pancreatic insulin production. When this compensatory mechanism fails, plasma glucose levels will rise and overt diabetes will develop.

A multitude of literature has firmly established the impact of modified dopaminergic transmission on glucose and insulin metabolism, yet several questions still remain unanswered. With the research described in this thesis, we sought to answer 2 main questions: is the altered dopamine signaling causally related to the development of diabetes? And, what is the mechanism underlying the ability of dopaminergic drugs to modify glucose metabolism? Knowledge of the developmental mechanisms of diabetes will hopefully assist in reducing morbidity and mortality by preventing the onset of diabetes as well as improving treatment.

In this chapter the major conclusions and implications of our findings are discussed in light of current knowledge.

Pharmacological modification of the dopaminergic system

To unravel the underlying mechanisms we examined the impact of DRD2 activation and inhibition on nutrient and energy metabolism. Inhibition of DRD2 by means of haloperidol and olanzapine induced glucose intolerance and insulin resistance (chapters 3-5) and activation of dopamine D2 receptors by means of bromocriptine led to improved insulin sensitivity (chapter 4). Although the results presented here show that activation and inhibition of dopamine D2 receptors lead to opposite metabolic profiles, the underlying mechanisms are distinct.

We showed in chapter 4 that subchronic treatment with bromocriptine leads to a reduction in body weight and fat mass, which is consistent with other experiments in rodents and humans[1-3]. The mechanism responsible for the decrease in body weight and fat mass is still unknown. The most straightforward explanation would be a reduction in energy intake and/or increase in energy expenditure, but, neither of these mechanisms occurred in our experiments. In accordance with our findings, Cincotta et al. showed that hamsters on bromocriptine treatment lost body weight and fat mass without alterations in food intake and energy expenditure[4]. Also, mice treated with bromocriptine displayed a significantly greater weight loss than pair fed mice[5,6]. Therefore, one must conclude that bromocriptine modifies adiposity and body weight via mechanisms other than food intake and energy expenditure.

As obesity and the associated increase in fat mass represent a significant risk factor for the development of insulin resistance[7], and loss of body fat and weight improves insulin sensitivity[8,9], the impact of bromocriptine on body weight and adiposity might participate in its positive effect on insulin sensitivity. However, several studies in humans show that bromocriptine beneficially alters the diabetic phenotype without implicating body weight and fat mass[10,11], indicating that alterations in adiposity are not necessarily involved in the positive action of the drug on glucose and insulin metabolism.

Bromocriptine also controls insulin secretion; in chapter 6 the drug acutely inhibits glucose-stimulated insulin secretion, which results in glucose intolerance. In agreement with this, mice injected with the DRD2 agonist cabergoline also acutely displayed glucose intolerance[12]. One can assume that suppression of insulin secretion leads to a diabetes-like phenotype. Initially, this assumption is true, as mice acutely develop glucose intolerance; however, in apparent contrast, we also showed that bromocriptine treatment for 2 weeks improved insulin sensitivity (chapter 4). This is in accordance with a wealth of literature showing that (sub)chronic bromocriptine treatment improves insulin secretion, glucose tolerance and insulin resistance in humans and animals[1,2,10,13]. To explain the discrepancy between acute and chronic treatment, we propose that bromocriptine promotes β-cell 'rest', leading to short-term deterioration and long-term improvement of glucose metabolism.

Beta-cell dysfunction is crucial in the development of diabetes; insulin resistance only progresses to overt diabetes when β-cells fail to secrete sufficient amounts of insulin to overcome whole body insulin resistance. This malfunction of β-cells is the corollary of an increased rate of apoptosis and changes in the intracellular pathway controlling insulin secretion. It has been hypothesized that the high glucose levels, fundamental in diabetes, might be, indirectly, responsible for β-cell degeneration by promoting insulin hypersecretion and consequently β-cell exhaustion and death[14,15]. Indeed, pharmacologically increasing insulin release for 48 h subsequently decreased insulin secretion in rats[16]. Accordingly, suppression of insulin secretion, preventing hypersecretion and death, might in the long-term, improve β-cell function. This concept has been verified in several experiments. Treatment of diabetic rats with the insulin secretion inhibitor diazoxide enhanced the diminished glucose-stimulated insulin response[17]. And, short-term treatment of diabetic patients with insulin secretion inhibitors attenuated the defective insulin release characteristic for type 2 diabetes[18,19]. Two mechanisms might explain the long-term beneficial impact of the initially deleterious impact of the suppression of insulin secretion: 1) inhibition of insulin secretion increases β-cell insulin stores, thereby enhancing the secretory capacity[17,20], and 2) inhibition of insulin secretion increases the number of organ specific insulin receptors leading to improved insulin sensitivity[21,22].

Finally, bromocriptine might also directly improve insulin sensitivity. In chapter 4 we showed that 2 weeks of bromocriptine treatment reduced insulin resistance. However, as up till now, no studies examining the acute impact of the drug on insulin action have been performed, it remains to be determined whether bromocriptine directly modulates insulin action or indirectly via its effect on insulin secretion[21,22].

The disruption of insulin action by inhibition of dopamine D2 receptors is achieved via other mechanistic routes than the improvement of insulin action by stimulation of D2 receptors. However, like bromocriptine, haloperidol treatment also participates in body weight regulation; although after subchronic treatment (2 weeks) body weight was not affected (chapter 4), after chronic treatment (12 weeks) body weight of treated mice was significantly increased compared to control mice (chapter 3). Keeping in mind that, due to the experimental setup of the latter study, food intake of haloperidol and control mice was identical, obviously the impact of haloperidol on weight is independent of alterations in food intake. In accordance with our findings, Pouzet et al. reported that an increased food efficiency, indicating an enhanced ability of food to increase body weight, was responsible for haloperidol induced weight gain[23]. In our experiments, mice treated with haloperidol for 1 week displayed a tremendous reduction in physical activity (chapter 4); in fact, this is a common phenomenon in rodents treated with antipsychotic drugs[24-26]. This reduced activity and concomitant reduction in energy expenditure might well account for the enhanced food efficiency and the body weight gain induced by the drug. If haloperidol indeed induces weight gain as we propose, an intriguing question is why we did not observe an increased body weight in mice treated with haloperidol for 2 weeks (chapter 4). Two explanations can be thought of: 1) the treatment period was too short to reveal differences in body weight. This would be in line with the chronic experiment in which alterations in body weight were not observed before the third week of treatment, or 2) haloperidol may have slightly, albeit not significantly, reduced food intake in the 2-week experiment, thereby preventing weight gain.

Besides its possible deleterious impact on insulin sensitivity via the development of obesity[7], the reduction in physical activity might also directly affect insulin sensitivity, independent of weight gain. It has consistently been shown that 6-10 days of bed rest, representing severe physical inactivity, impairs insulin sensitivity in healthy man without affecting body weight[27-29]. Also in trained volunteers refraining from exercise for 10-14 days, representing a milder protocol for inactivity, insulin resistance is observed, again without alterations in body weight and fat mass[30,31]. Typically, this inactivity induced insulin resistance is restricted to tissues responsible for glucose-uptake, as glucose production remains adequately suppressed by insulin[27,28,32]. It has been suggested that a reduction in GLUT4 expression might underlie the inactivity

induced impairment of glucose uptake[31], but more research is warranted to confirm this.

In addition, haloperidol, and other DRD2 antagonists, might directly affect insulin sensitivity, independent of their impact on physical activity and body weight gain. In fact, it is known that several antipsychotic drugs, other than haloperidol, are able to acutely induce insulin resistance[26,33-35]. This insulin resistance seems to involve both glucose uptake and glucose production[33-35], although the acute impact of antipsychotic medication on glucose production is not always observed[26]. Interestingly, the antipsychotic drug induced inability of tissues to take up glucose during hyperinsulinemia seems largely confined to muscle tissue, as glucose clearance by adipose tissue is even enhanced[26]. Even though the direct impact on insulin sensitivity has not yet been confirmed for haloperidol, the drug does acutely impair glucose tolerance[36,37]. This implies that haloperidol is able to reduce insulin secretion and/or promote insulin resistance. As the glucose intolerance was accompanied by elevated insulin levels[36], defective insulin secretion can not (solely) explain the glucose intolerance. This provides evidence that haloperidol, like other antipsychotics is able to acutely decrease insulin sensitivity.

Finally, haloperidol might also impair insulin secretion; after 10 weeks of treatment, haloperidol and control mice had, despite significantly increased glucose levels in the former, similar insulin levels during a glucose tolerance test (chapter 3). This indicates an insulin secretion malfunction. Likewise, the low basal insulin levels in the face of elevated basal glucose levels observed in these mice after 12 weeks of drug treatment, confirm the hypothesis that β-cells are unable to produce sufficient amounts of insulin. These findings are in accordance with studies in DRD2 deficient mice, which also show inappropriately low insulin levels during an i.p. glucose tolerance test[12]. In vitro experiments with isolated islets from these mice showed that glucose was unable to stimulate insulin secretion from these islets compared to islets from wt mice. Further examination of the pancreata of DRD2 deficient mice revealed a reduced β-cell mass and insulin concentration[12]. According to these results it is conceivable that in our chronically treated haloperidol mice, the malfunctioning insulin secretion is due to a reduced β-cell mass and/or intracellular β-cell defects. The mechanism underlying the deregulation of β-cell function has not been resolved yet, but it has been suggested that DRD2 activation is essential for β-cell proliferation[12]. Consequently, chronically blocking DRD2 could reduce β-cell proliferation and eventually lead to a diminished β-cell mass. Alternatively, one might speculated that, in analogy with the hypothesized impact of bromocriptine on insulin secretion, haloperidol may initially promote glucose-stimulated insulin secretion, which may lead to insulin hypersecretion and consequently to β-cell damage and death. Several papers document a reduced responsiveness of β-cells towards insulin secretagogues following prolonged stimulation of

insulin secretion[16,38], confirming the last part of the hypothesis. The initial part though, the acute effect of haloperidol on insulin secretion, remains to be verified as the literature on this subject is controversial. In an in vitro study by Best et al. haloperidol induced a depolarization of β-cell membrane potential and, although this might be expected to enhance insulin secretion, such effect could not be detected[39]. Two other studies reported a diminished and an unaltered insulin secretory response of β-cells following incubation with haloperidol[40,41].

All together, we have provided evidence that modulation of glucose homeostasis by activation or inhibition of dopamine D2 receptors is achieved via different mechanistic routes. Presumably, bromocriptine mainly improves glucose metabolism by suppressing insulin secretion which, paradoxically, leads to enhanced insulin action. Weight reduction, as a result of bromocriptine treatment, might additionally improve insulin sensitivity, but it is not a prerequisite for the beneficial impact of the drug. Haloperidol, on the other hand, most likely disrupts physiological glucose metabolism by reducing physical activity, which, directly, or via weight gain, reduces insulin sensitivity. In addition, the drug probably also directly promotes insulin resistance and gradually impairs insulin secretion.

Dopaminergic system and the aetiology of diabetes

With the experiments described in this thesis, we also wanted to gain more insight into the role of dopaminergic neurotransmission in the course of diabetes development. Several cross-sectional studies suggest that alterations in dopaminergic neurotransmission are involved in the pathogenesis of type 2 diabetes. In obese humans and insulin resistant animals the expression of dopamine D2 receptors in certain brain areas is reduced[42-45]. In obese humans the decrease in dopamine D2 receptors is even inversely related with BMI[42]. And, in brains of diabetic patients and type 2 diabetic animal models, increased dopamine levels are measured[46-48]. As cross-sectional research does not provide details about the cause-effect relationship, two hypotheses, based on the observations above, can be postulated: 1) altered dopaminergic neurotransmission is the *cause* of metabolic derangements or 2) altered dopaminergic neurotransmission is the *consequence* of metabolic derangements.

Considering the indisputable positive impact of DRD2 activation on glucose and insulin metabolism and the detrimental effect of blocking DRD2, described in chapters 3-5 and discussed above, the first hypothesis is more likely. This hypothesis requires that components involved in dopaminergic signaling are altered prior to the initiation of metabolic derangements. Genetic variations of dopaminergic genes may be responsible for the initial alterations. This is supported by the observed association between DRD2 polymorphisms which diminish dopaminergic transmission[49,50] and disturbed energy homeostasis[51-54].

However, as diabetes is also associated with obesity[7], reduced physical activity[55], aging[56], an altered dietary pattern[57-60] and the use of antipsychotics[61,62], we proposed that the initial modifications in dopaminergic activity might also be triggered by nutritional, environmental, pharmaceutical or physiological factors.

In chapter 2 we examined the hypothesis that high fat feeding, which is a well-recognized trigger for the development of a diabetes-like phenotype in rodents, induces these metabolic anomalies via modifications in dopaminergic neurotransmission. In contrast to our hypothesis, though, wt C57Bl6 mice, maintained on a high fat diet for 4 weeks, were insulin resistant compared to control mice without detectable alterations of dopaminergic features. Consequently, we concluded that the reduced dopaminergic neurotransmission observed in obese humans and animals is not due to dietary factors. There are several ways to explain the discrepancy between the literature, showing an altered dopaminergic phenotype in obese animals and humans and the absence of dopaminergic alterations in our experiment.

We hypothesized that nutritional cues will diminish dopaminergic action, thereby inducing insulin resistance, but, we did not consider the existence of dopaminergic gene variations that might be present in our mice. However, these polymorphisms could alter dopaminergic action, which might set the stage for high fat diet induced insulin resistance. This is supported by the finding that body weight gain in schizophrenic patients on antipsychotic drug treatment is associated with certain DRD2 gene variations[63,64]. Also, compared to diet-resistant rats, rats prone to become obese on a high fat diet already display alterations in dopamine metabolism when still maintained on a regular low fat diet[65,66]. In addition, already prior to the onset of food intake and body weight alterations, the expression of DRD2 in the striatum of obese Zucker rats is reduced compared to lean Zucker rats[67]. These observations strongly suggest that genetic variations in dopaminergic parameters determine the susceptibility of individuals to develop an unfavorable metabolic phenotype in response to pharmacological or nutritional cues. One may even speculate that these dopaminergic variations are a prerequisite for the development of metabolic alterations. This genetic predisposition might explain why only some rodents develop massive weight gain on a high fat diet (DIO; Diet Induced Obese) and others remain relatively lean (DR; Diet Resistant)[68-70].

If this theory is true, it is understandable that we found metabolic, but not dopaminergic, differences between the mice maintained on a high vs. low fat diet. Rodents prone to become obese (DIO prone) or remain lean (DR prone) on a high fat already have a different dopaminergic profile when still on the control diet[65,66]. This suggests that, in a random population of rodents, various dopaminergic phenotypes are present. We showed in chapter 4 that some C57Bl6 mice become more obese and insulin resistant on a high fat diet than

others, so it is possible that the C57Bl6 mice in our experiment initially already have different dopaminergic phenotypes. We believe this might be true. As we divided our mice in chapter 2 randomly into a high and low fat group, both groups could have contained mice with a 'normal' dopaminergic phenotype as well as mice with a, genetically-determined, 'deterimental' phenotype. It goes without saying that if both phenotypes were equally represented in both the high and low fat group, there would be, on average, no measurable difference in dopaminergic parameters between these groups. The corollary of the presence of these different dopaminergic profiles in the high fat group should have been the development of different degrees of weight gain and insulin resistance. Unfortunately, due to the small sample size, we were unable to divide the high fat mice into DIO and DR mice according to their dopaminergic and metabolic phenotype. So, obviously, more research is warranted to confirm this hypothesis.

If, however, the first assumption that dopaminergic neurotransmission is the *cause* of metabolic derangements is incorrect, is it then possible that dopaminergic alterations are the *consequence* of changes in the hormonal environment in diabetic individuals? In other words, is it possible that in our experiment dopaminergic alterations would have developed *after* insulin resistance was established? This might be true. Hyperglycemia, a hallmark of diabetes, promotes elevated brain dopamine levels[71-73]. NPY, which's levels are elevated in obese and diabetic individuals[74], stimulates dopamine output[75]. PYY (3-36) suppresses dopamine release[76] and its levels are reduced in obese subjects[77]. Leptin also reduces dopamine output[78,79] while chronic obesity is characterized by a resistance to the actions of this hormone[80]. In apparent contrast, insulin acutely increases dopamine uptake by promoting the surface expression of the dopamine transporter[81] and chronic insulin stimulation upregulates dopamine transporter mRNA[82]. In conclusion, these results indicate that disturbances of several internal regulators of energy balance might account for the alterations in dopaminergic neurotransmission observed in obese diabetic animals and humans. Yet, the physiological role and relevance of these processes in the course of obesity and diabetes development remain to be determined.

All together, we presented evidence that alterations in dopaminergic signaling may be either cause or consequence of the diabetic phenotype. Polymorphisms in dopaminergic genes may determine the susceptibility of a subject to develop obesity and diabetes in response to nutritional of pharmacological cues. On the other hand, diabetes-associated disturbances of hormone levels, or action, may promote alterations in dopamine homeostasis.

Humans versus rodents

Interestingly, in our experiments we observed a discrepancy between the impact of haloperidol in humans and mice. In humans haloperidol did not

modify glucose and insulin metabolism (chapter 5) whereas in mice haloperidol clearly induced glucose intolerance and insulin resistance (chapter 3 and 4). Several explanations can be thought of: 1) different dose, 2) different mode of administration, 3) different treatment period and 4) species-specific sensitivity to the effect of haloperidol.

First, the difference in dose; in mice we used 1 mg/kg/day, whereas the human volunteers were treated with 3 mg haloperidol per day, which corresponds to 0.04 mg/kg/day given the average weight of the subjects (~75 kg). When comparing drug doses between humans and rodents, though, one must take into account that the metabolism of drugs in rodents is faster than in humans, resulting in a, in general, 4 - 6 times shorter half-life of drugs in rodents[83]. So for rodents, the dose comparable to the one used in humans would be 0.16 - 0.24 mg/kg/day. The dose given to the human volunteers is in the low range of doses prescribed to schizophrenic patients, whereas the dose given to the mice is in the high range of doses used to treat schizophrenia. Possibly, this difference in medication dose can explain the dissimilarities in efficacy of haloperidol in mice and man. Experiments performed in rodents using a low dose of haloperidol (0.25 mg/kg/day), equivalent to the dose used in our human study, showed no impact of the drug on glucose metabolism after 7 and 28 days of daily injections, although, interestingly, glucose intolerance was observed 1 hour after the first injection[36].

Another variable in these studies is the mode of drug administration which might further affect the plasma levels of the drug. In our experiments mice received haloperidol through subcutaneous implanted pellets, while human individuals received haloperidol tablets. Two issues regarding the mode of administration are relevant in this discussion: the frequency of drug administration (continuous vs. once daily) and the route of drug entry (subcutaneous vs. oral). As described above, the half-life of drugs in rodents is considerably shorter than in humans. Specifically, in humans, the half-life of haloperidol is 12-36 hours; in rodents, the half-life is only approximately 1.5 h[83]. According to these figures, in humans a 'single administration a day' regiment is sufficient to achieve a relatively stable plasma concentration of haloperidol and level of DRD2 receptor occupancy throughout the day. The short half-life of haloperidol in mice though, imposes that the drug should be administered approximately 8 times a day in order to achieve a similar stable drug concentration and receptor occupancy. The most practical way to ensure stable plasma haloperidol concentrations in mice is to use pellets or minipumps continuously releasing the drug. So, most likely, the frequency of drug delivery does not explain the discrepant impact of haloperidol in humans and mice, but the route of drug delivery may. Compared to subcutaneous drug administration, which we used in our mice experiments, the efficacy of orally administered drugs is limited by the so-called 'first pass effect'. Orally ingested drugs are

absorbed by the gastrointestinal tract and are transported, via the portal vein, to the liver before entering the systemic circulation. The liver metabolizes part of the drug, in case of haloperidol, into inactive compounds, thereby limiting the bioavailability of the drug. So, the oral delivery route may have further amplified the impact of the different doses used in the human and mice studies.

Another variable was the treatment period; the volunteers were treated for 8 days (chapter 5), while the mice were treated for 14 days (chapter 4) or 12 weeks (chapter 3). One can propose that 8 days of treatment is too short for metabolic alterations to emerge, yet, this is most likely not true. Haloperidol already induces glucose intolerance as soon as 1 hour after injection in mice[36]. This strongly suggests that 8 days of haloperidol treatment should suffice to uncover the metabolic consequences of treatment if any were present.

Finally, a species-specific sensitivity should also be considered. The existence of such species-dependent sensitivity to antipsychotic drugs is perhaps best illustrated by the effect of those drugs on weight gain. In contrast to the human situation where both males and females develop obesity in response to antipsychotic drug treatment, in rats, only females seem to be susceptible for the weight inducing ability of these drugs[23,84]. Up till now, only one group, using a specific treatment protocol, has been able to induce obesity in male rats in response to olanzapine[85]. Also the impact of haloperidol on weight gain is different in rats and humans; low concentrations of haloperidol already trigger body weight gain in female rats[23], while this drug is associated with no, or very limited weight gain in the humans[86,87]. This discrepant body weight regulation in response to antipsychotic drugs suggests that glucose metabolism might also be differentially affected in humans and rodents, but to confirm this, thorough dose-response experiments should be performed in both species.

In conclusion, the absence of metabolic consequences in haloperidol treated humans in spite of the insulin resistance observed in mice treated with haloperidol is most likely due to a combination of factors: the bioavailability of the drug, determined by the drug dose and the route of administration, together with the species-specific sensitivity to the drug.

Conclusion

With the experiments described in this thesis we attempted to unravel the intricate relationship between diabetes and DRD2 mediated dopaminergic transmission. We provided evidence that although both DRD2 agonistic and DRD2 antagonistic drugs affect glucose metabolism, the mechanistic routes are distinct. Unlike bromocriptine, which beneficially affects insulin action by, paradoxically, suppressing insulin secretion, haloperidol disturbs insulin action by diminishing physical activity and directly disrupting insulin sensitivity.

We also discussed that dopaminergic dysfunction might be cause or consequence in the aetiology of diabetes. Genetic variations in dopaminergic

genes, leading to diminished dopaminergic transmission, may predispose individuals to develop a diabetes-like phenotype in response to physiological or pharmacological cues. Alternatively, dopaminergic transmission may be disturbed by diabetes-induced alterations in the hormone profile.

Although caution is warranted when extrapolating the results of drug experiments obtained in animals to humans, especially with regard to the dose-effect relationship, we believe the general mechanisms we observed in these animals are also applicable to humans.

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