Type 1 diabetes, glucocorticoids and the brain: a sweet connection

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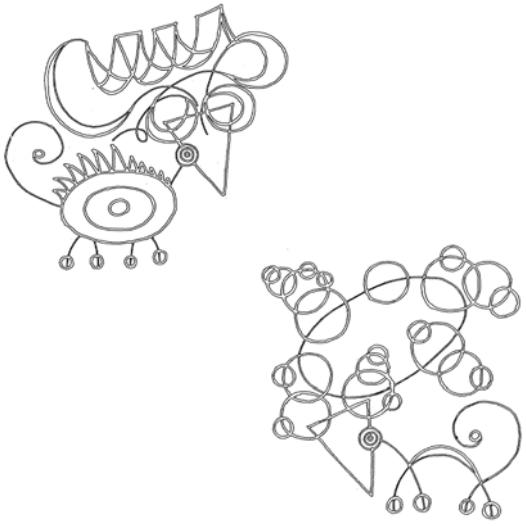
CHAPTER 1

GENERAL INTRODUCTION



Outline

1. Type 1 diabetes

The earliest known record of diabetes dates from 1552 B.C., when the 3rd Dynasty Egyptian papyrus by physician Hesy-Ra mentioned polyuria (frequent urination) as a symptom of the disease. Over the years, diabetes was described and studied by Egyptians, Greeks, Chinese, Indians, English, Frenchs, Germans, Czechs, Italians, Canadians, Americans, and others. However, was not until the last century, in 1959, that the two major types of diabetes were recognized: type 1 (insulin-dependent) diabetes and type 2 (non-insulin-dependent) diabetes.

Nowadays diabetes affects over 150 million people worldwide with this number expected to double by 2025; about 90% cases of diabetes are type 2 (Zimmet *et al*, 2001). However, the fraction of type 2 diabetics in different parts of the world varies substantially, almost certainly for environmental and lifestyle reasons, though these are not known in detail. Since type 2 diabetes is not the topic of this thesis, it will not be described at length.

Diabetes mellitus type 2, also called type 2 diabetes, Non Insulin Dependent Diabetes Mellitus (NIDDM) or Adult diabetes, is a metabolic disorder that is primarily characterized by insulin resistance, relative insulin deficiency, and hyperglycemia, and is presently of unknown etiology although there is a strong inheritable genetic connection. About 55% of type 2 are obese (Eberhart *et al*, 2004) -chronic obesity leads to increased insulin resistance that can develop into diabetes, most likely because adipose tissue is a source of chemical signals (hormones and cytokines). Conversely, type 2 diabetes causes obesity (Camastra *et al*, 1999). Additional factors found to increase risk of type 2 diabetes include aging (Jack *et al*, 2004), high-fat diets (Lovejoy, 2002) and a less active lifestyle (Hu, 2003).

Diabetes mellitus type 1, also known as type 1 diabetes (T1D), Insulin Dependent Diabetes Mellitus (IDDM) or Juvenile Diabetes, is an autoimmune disease that results in the permanent destruction or damage of insulin producing beta-cells in the islets of Langerhans of the pancreas. Destruction of these cells leads to insulin deficiency. Therefore, T1D is lethal unless treatment with exogenous insulin via injections replaces the missing hormone. Although, the clinical consequences of the disease have been extensively investigated, the exact cause(s) of T1D are not yet fully understood. Genetic and environmental factors have been suggested to contribute to the etiology of T1D along with other factors (see Box 1 on page 14). So far, the research core on diabetes has focused on the peripheral endocrinology and nervous system. Nowadays, the impact of diabetes on the central nervous system (CNS) is highly recognized but it was not always the case until few decades ago. To study disease initiation, progression, and treatments without exposing humans to unnecessary and potentially unethical risks animal models have been developed. Animal models have contributed important knowledge regarding the study of diabetes. The physiology of mice, rats, and other animals is remarkably conserved in comparison to the human condition. Broad spectrum of animal models of T1D have become available over the last 40 years. They comprise spontaneous models, in which disease develops unprovoked, and experimental models induced by various types of intervention (Table 1, page 15).

1.1. Animal models of diabetes

1.1.1. Spontaneous Models

The two major models used are the so-called Bio Breeding (BB) rats and the Non Obese Diabetic (NOD) mice, which develop the disease with similarities to human T1D. These animals derived from inbreeding over many generations by selecting for hyperglycaemia. As a result, many genes and phenotypes have been enriched, but not all will be relevant to the pathophysiology of diabetes, either in rodents or in humans. It is noteworthy that a main advantage of these models is the possibility to study the pre-diabetic state, which is impossible in humans. Other models comprise the Long Evans Tokushima Lean (LETL) rat and the LEW.1AR1/Ztm- iddm rat (Table 1). For sake of clarity, only the model used in the current thesis will be described below.

1.1.1.a. The NOD mouse

The NOD mice were first used in the study of cataract development (i.e. JcI-ICR mouse) (Makino *et al*, 1980). Insulitis, which is the lymphocytic infiltration of the islets of Langerhans, is present by the time mice reach 4-5 weeks of age. This state is followed by beta-cells destruction and ultimately leads to a drastic decrease in circulating insulin. In the pre-diabetic state (4-5 weeks of age), NOD mice show lower glycemia and higher insulinemia in response to a glucose tolerance test compared with C57Bl/6 control strain (Amrani *et al*, 1998). Frank diabetes typically begins between 12 and 30 weeks of age. Unlike human T1D, ketoacidosis (metabolic acidosis is caused by high concentrations of ketone bodies and breackdown of fatty acids) is relatively mild and affected animals can survive for weeks without the administration of insulin. In addition and in contrast to the findings of most studies in humans, there is a larger gender difference with 80% of females, but only 50% of males developing diabetes in some colonies (Atkinson and Leiter, 1999). This variation is not surprising knowing that sex steroids are part of the mechanisms underlying the well-recognized immune sexual dimorphism, which is particularly evident in autoimmune diseases (Ansar Ahmed *et al*, 1985). Moreover, NOD diabetes can be modulated not only by multiple immunotherapeutic agents (Bach 2002), but also by various other factors, including melatonin, insulin growth factor-1 (IGF-1), leptin, insulin and drugs modulating its secretion or sensitivity, and environmental factors such as temperature fluctuations, variations of protein and carbohydrate intake, and stress.

Stressful life events and diabetes onset linkage have been reported in clinical and experimental studies (Homo-Delarche *et al*, 1991; Djarova and; Dube, 1998.). As part of the endocrine response to stress, glucocorticoids exert well-known anti-inflammatory and immunosuppressive actions but also act as counterregulatory hormones inducing hyperglycemia. Therefore, in T1D, glucocorticoids might have both potentially beneficial and deleterious effects.

1.1.2. Experimental models

Most of the experimentally induced models correspond to highly artificial situations far from the conditions in which spontaneous disease develops. However, they have made possible remarkable progresses in understanding the pathogenesis of T1D. Chemically-induce, transgenic, and immunomanipulated mice are among these models. In the following section, the experimental model used in the present thesis is described.

1.1.2.b. Chemically induced T1D: Streptozotocin-induced diabetes mice

Pharmacological methods of inducing T1D by damaging the pancreas also exist. These include the administration of toxins such as streptozotocin (STZ) (Junod *et al*, 1969) and alloxan. Streptozotocin is a glucosamine–nitrosourea compound isolated from *Streptomyces achromogenes* with broad-spectrum antibiotic and anti-neoplastic activity (Bono *et al*, 1976). It is a powerful alkylating agent that has been shown to interfere with glucose transport (Wang and Gleichmann, 1998), glucokinase function (Zahner and Malaisse, 1990) and induce multiple DNA strand breaks (Bolzan and Bianchi, 2002). It is taken up into the insulin-producing beta-cells of the islets of Langerhans via the GLUT-2 glucose transporter (Schnedl *et al*, 1994). The GLUT-2 glucose transporter is absent at the blood–brain barrier (Kumagai, 1999), thus excluding direct effects of STZ on the brain following systemic administration. A single large dose of STZ can produce diabetes in rodents, probably as a result of direct toxic effects. Alternatively, multiple small doses of STZ are used (e.g. 40 mg/kg on five consecutive days) to study the immunological pathways that lead to insulitis and cell death (Mensah-Brown *et al*, 2002; Holstad and Sandler, 2001). STZ-diabetic rodents are hypoinsulinaemic, but do not require insulin treatment to survive. Blood glucose levels typically are 20-25 mmol/l, which is 5 fold over normal concentration. In rodents, hyperglycemia induces an insulinopenic (lack of insulin) diabetes in which immune destruction plays a role, as in human T1D.

Box 1: Etiology of type 1 diabetes

The origin of the autoimmune process that leads to type 1 diabetes (T1D) involve genetic predisposition (as T1D is known as a hereditary disease on basis of the relatively high rate of familial transmission (1)) and environmental factors, and their interactions, which creates the conditions required for disease onset. The patterns of familial transmission, combined with data from animal models, indicate that the determinism of T1D is polygenic and multifactorial. The search predisposition genes is complex, especially as most if not all predisposition genes appear to be basically "normal" i.e. without mutations or deletions. A fortuitous combination of these genes, together with permissive or triggering environmental factors, provokes the disease. Each of these genes may be present in a large proportion of healthy subjects (notably the patient's nondiabetic relatives).

Evidence for the role of environmental factors:

Several lines of evidence point to a major role of environmental factors in the pathogenesis of T1D. First, more than 60% of identical twins are discordant for the disease, and it is quite unlikely that this is due to differential somatic rearrangement of T cell receptors. Second, disease frequency varies enormously from country to country (2), and these differences cannot simply be explained by ethnic genetic differences since migrants from countries with a low T1D frequency to countries with a high frequency are more susceptible than their compatriots (3). Intriguingly, northern countries are more exposed to the disease than southern countries (2); it will be critical to discover the factor(s) responsible for this North/South gradient. Third, a number of apparently nonimmunological interventions can increase or decrease the disease rate in animal models: specific diets (low essential fatty acid (4) or protein intake (5, 6)) and several viral infections (7-11) can reduce disease susceptibility in spontaneous models of T1D, the NOD mice and the BB rats, while Kilham's virus (12) and cow's milk (13,14) can increase it in BB rats. These factors, particularly viral infections, probably explain the variations in disease frequency found between NOD colonies (15). Not only do environmental factors seem to influence T1D onset, they can also apparently alter the course of the disease. These factors can be shared by the whole population (climatic factors, hygiene, etc.), or by a given family (e.g. eating habits), or be specific to the individual (e.g. travels and sexual partners). Several studies have focused on many potential environmental factors involved in the etiology of T1D, such as viruses (16-18), bacteria's (19-22), toxic agents (23, 24), food constituents (5, 6, 13, 14), stress (25-28). These factors essentially modulate the expression of predisposing genes, either positively (predisposing factors) or negatively (protective factors). In the case of triggering factors, disease onset is directly related to the encounter with the environmental factor (usually single and limited in time), which can then be considered as the cause of the disease. In the "modulation" hypothesis, the disease can only appear in the fraction of the population at genetic risk and it is on this population that environmental factors (usually multiple and chronic) exert their positive or negative effect. The available data suggests that T1D is of the second type.

Table 1. Animal models of type 1 diabetes

Spontaneous models
1. NOD mouse: inbreed strain. Develop Type 1A-Immune Mediated Diabetes. Autoimmune etiology that is heavily influenced by both genetics and environment (1). 2. BB rat: inbreed strain. Diabetes in BB rats is also an autoimmune disorder. Substrain BB/Wor has profound T-cell lymphopenia (condition in which there exists an abnormally low number of lymphocytes in the blood) (2). 3. Long Evans Tokushima Lean (LETL) Rat: autoimmune T1D (3) 4. LEW.1AR1/Ztm- iddm rat: autoimmune T1D, spontaneous mutation within a Mhc-congenic LEW.1AR1 colony (4).
Experimental models:
Transgenic 1. T Cell Receptor (TCR) Tg (transgenic) Mouse: many cell clones isolated from the spleens of diabetic NOD mice, pancreas of pre-diabetic NOD, islet-transplanted diabetic NOD mice, and from islets of NOD mice. Many of these clones have been utilized to produce TCR transgenic (Tg) mice on various backgrounds (5) 2. "Humanized" Mice: transgenic expression in mice of human genes (6). *Chemically-induced* 1. alloxan (7) 2. streptozotocin (8) *Immunomanipulation* 1. thymectomy performed within 2 days after birth can induce a flourishing state of autoimmunity in mice (9). 2. adult thymectomy and sublethal irradiation (10, 11). 3. athymic rats with transfer of normal spleen cells (12).

2. Hypothalamic-pituitary-adrenal axis and type 1 diabetes

2.1. HPA axis

The hypothalamic-pituitary-adrenal (HPA) axis refers to a complex set of homeostatic interactions between the hypothalamus (brain area); the pituitary gland (structure located below the hypothalamus), and the adrenal glands (small pair of pyramidal organs located on top of the kidneys). The HPA axis regulates responses to stress and modulates various body processes including growth, metabolism, immune response, mood, reproduction, sexuality, and energy balance. The core of the HPA axis is the paraventricular nucleus of the hypothalamus (PVN). The PVN contains neuroendocrine neurons, the so-called parvocellular neurons, which synthesize and secrete vasopressin (AVP) and corticotropin-releasing hormone (CRH). These two peptides can act in synergy on the anterior lobe of the pituitary gland to stimulate the secretion of the adrenocorticotropic hormone (ACTH) from corticotrope cells. In turn, ACTH enters peripheral circulation where it reaches the adrenal cortex to induce glucocorticoid hormones production (cortisol in humans, corticosterone in rats and mice). Glucocorticoids exert a negative feedback on the PVN and pituitary to suppress CRH and ACTH production, respectively.

Corticosterone is a major stress hormone and has effects on wide arrays of tissues in the body, including the brain. In the brain, corticosterone acts via two types of receptors - mineralocorticoid receptor (MR) and glucocorticoid receptor (GR). These receptors are widely expressed throughout the brain by many different types of cells including neuron and glia. MR and GR have different affinities to glucocorticoids (GCs) with MR showing a greater affinity (10 fold higher) than GR. As a consequence, MR is fully occupied under basal circulating levels whereas GR becomes occupied only when glucocorticoids levels rise above normal. One important target of glucocorticoids is the hippocampus, an area of the limbic system that plays a critical role in memory, learning and spatial navigation. This structure is a major modulator of the HPA axis; hippocampal MR controls the inhibitory tone of this limbic structure on the HPA axis in terms of basal reactivity (Reul *et al*, 2000). This effect of GCs via MRs is modulated by GRs that become progressively occupied after stress and during the circadian rise of GCs. Therefore, predominant MR activation maintains hippocampal excitability and, through inhibitory projections to the PVN, basal HPA activity. Conversely, with rising GCs concentrations, GR activation suppresses the hippocampal output, resulting in a disinhibition of PVN neurons (de Kloet *et al*, 1998). In summary, a deficiency in MR is predicted to allow more GC release, thus leading to more pronounced GR-mediated effects. Therefore, the functions mediated by both receptor types are linked, and the balance in MR- and GR-mediated effects is important in the HPA regulation.

2.1.1. Stress concept

Stress is the disruption of homeostasis through physical or psychological stimuli. Internal

or external potential disturbances (stressors) activate two systems that serve to normalize the disturbed functions: the rapid sympatho-adrenomedullar system and the slow-acting HPA axis. The activation of the sympathetic branch result in the release of stress hormones including adrenaline from the adrenal medulla. Therefore, activation of noradrenergic neurons leads to temporarily elevated noradrenaline (NA) levels in specific areas of the brain resulting in functional changes of neurons carrying NA receptors. Activation of the HPA system, leads to increase GCs release from the adrenal cortex, which in turn will act in the brain at those sites where its receptors are enriched (Figure 1) (Joëls *et al*, 2007).

Sympathetic nervous output produces the fight-or-flight response, causing the body to divert blood flow to large muscles as the body prepares to run away from or fight something. Lower blood flow is then directed to the digestive system and other organs that do not assist in flying or fighting. Some stressors can cause continual sympathetic nervous system activation with very little opportunity for the parasympathetic nervous system to be activated. The activation of the parasympathetic system stops the fight-flight responses.

Experimental studies have investigated many different types of stressors, and their effects on the HPA axis in many different contexts. Analysis of the literature suggests that different classes of stressor employ different stress circuits. Severe physiologic ("systemic") stress appears to trigger brainstem/circumventricular organ systems that project directly to the PVN. In contrast, stressors requiring interpretation with respect to previous experience ("processive" stressors) reach the PVN by way of multisynaptic limbic pathways. Stressors of the latter category may thus require interaction with homeostatic information prior to promoting an HPA axis response. The HPA stress response thus appears to be a product of both the physiologic importance of the stimulus and the specific pathways a given stimulus excites (Herman *et al*, 1996). The activation of the HPA axis will ultimately trigger GCs secretion. In healthy condition this highly reactive system will turns on and off its responses to stressors. However, if adaptation to stress fails, the stress system responds slowly, or the stress reactions persist, circulating GC levels remain elevated for a prolonged period of time and an enhance vulnerability to disease for which the individual is predisposed may occur (de Kloet and de Rijk, 2004).

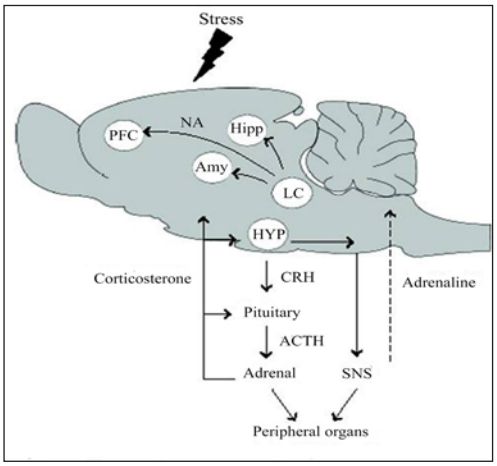


Figure 1: *Brain regions activated after stress exposure (Amy: amygdala, Hipp: hippocampus, PFC: prefrontal cortex) and output of these areas through the hypothalamus (HYP). A resulting activation of the fast acting sympatho-adrenomedullar system (right) and the slower acting HPA axis system (left) will affect the function of peripheral organs and feed back to the brain via adrenaline and corticosterone, respectively. Adrenaline will finally rise central release of noradrenaline leves from the locus coeruleous (LC), reaching again the amygdala, prefrontal cortex and hippocampus among other areas. Corticosterone will act on brain areas where its receptors are enriched.*
SNS = sympathetic nervous system; ACTH = adreno-corticotropin hormone; CRH = corticotropin releasing hormone.
Reprinted with permission from Joëls and Krugers, Neural Plasticity 2007.

2.2. Hypothalamic-pituitary-adrenal axis alterations in type 1 Diabetes

When T1D develops, the insulin producing beta-cells in the islets of Langerhans from the pancreas are destroyed (Figure 2A). Hence, hyperglycemia develops; however, since insulin controls glucose intake, lack of insulin creates of state of cellular starvation. Both hyperglycemia and cellular starvation coexist and, under certain conditions such as T1D, can generate metabolic stress.

The metabolic stress will activate the HPA axis (Figure 2B) in an attempt to restore homoeostasis and recover from metabolic disturbance. The HPA axis activation will ultimately raise basal plasma GCs levels, which will be followed by the shut down of the HPA axis via GCs negative feedback.

However, previous reports in T1D animal models showed GR downregulation in the hippocampus (Tornello *et al*, 1981) and HPA axis hyperactivity (Chan *et al*, 2001 and 2002) (Figure 2C). These results explain, in part, the chronic hypercorticism observed in T1D patients and animals. On the other hand, GCs exert an hyperglycemic effect, inhibiting cellular glucose uptake in the periphery and also in neurons and astrocytes of brain regions such as the hippocampus (Munck *et al*, 1984; Sapolsky, 1992). Therefore, pre-existing hyperglycemia will become chronic state. In this way, continuous metabolic alterations will contribute to the defective shut-off of the stress response. When HPA axis activity is chronically elevated or managed inefficiently, various forms of pathophysiologies are promoted, such as dysfunction of the hippocampus (McEwen 1998; Sapolsky, 1992).

In summary, in agreement with published reports, diabetic animals showed a sustained stimulation of the HPA axis, with elevated basal plasma GC levels. In addition, adrenal hypertrophy, and spleen and thymus atrophy was found; characteristics also of long-lasting exposure to high amounts of GCs (Scribner *et al*, 1993; Oster *et al*, 1988; Bellush, *et al*, 1991). The observation that diabetic animals show a poor shut-off of the stress response (Magarinos and McEwen 2000; Chan *et al*, 2001; McEwen *et al*, 2002) suggests insensitivity to feedback mechanisms and is consistent with the reported resistance to the dexamethasone suppression test (Scribner *et al*, 1993) and downregulation of GR levels in the hippocampus of STZ rats (Tornello *et al*, 1981). In conclusion, processes to adapt to the metabolic imbalance will be created in T1D, involving poor glucose homeostasis, chronically elevated GC levels, increased HPA axis reactivity, and metabolic adjustments. Extreme metabolic adjustments can induce and significantly accelerate hippocampal remodeling, increasing its vulnerability to diabetes, and cognitive dysfunctions.

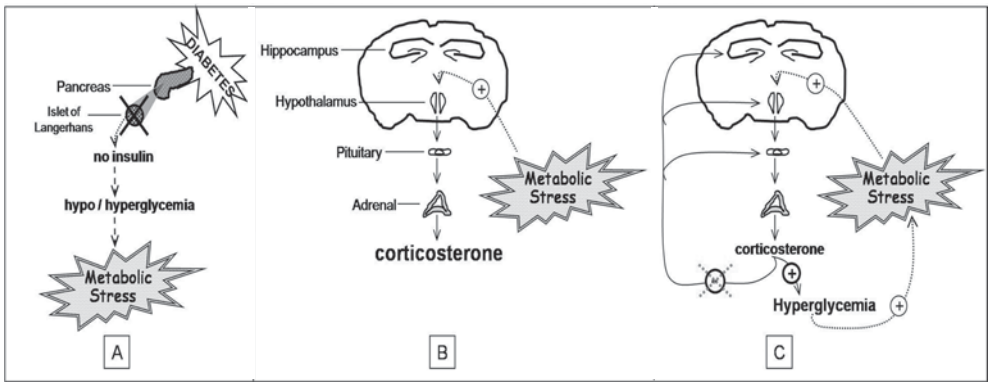


Figure 2. *HPA axis alterations in type 1 diabetes. Metabolic stress develops when insulin-producing cells destroy leads to hyperglycemia (A). As a consequence, the activation of the HPA axis triggers an increase in corticosteroids levels (B). The chronic high corticosterone concentration, on one hand exacerbates glucose secretion, and on the other hand acts on the HPA axis in order to shut-off the stress response and restore the homeostasis (C). According to the literature, in type 1 diabetes this response is disrupted.*

3. Impact of Type 1 Diabetes

3.1. Central Nervous System

Diabetic nephropathy, neuropathy, and retinopathy are traditionally considered the late complications of diabetes, whereas CNS was believed to be spared from diabetic complications. However, substantial evidence from clinical and experimental studies demonstrates that diabetes causes primary disease duration-related impairments in CNS function besides secondary sequelae of cerebrovascular events mediated by diabetic macrovascular disease (Li *et al*, 2002; Sima *et al*, 2004). In particular, hyper- and hypoglycaemic episodes may result in acute cerebral dysfunction (Biessels *et al*, 1994; Cryer *et al*, 1994). The consequences of these acute insults to the brain are well recognized. However, there is little knowledge about functional and structural cerebral alterations that develop more insidiously in diabetes. Long-term effects of diabetes on the brain are manifested at the structural, neurophysiological and neuropsychological level. The emerging view is that the diabetic brain features many signs that are best described as accelerated aging.

In diabetic rodents, structural abnormalities including synaptic and neuronal alterations, degeneration, neuronal loss, glycogen accumulation, dilated and fragmented endoplasmic reticulum, increased microtubuli, and irregular nuclei have been demonstrated (Bestetti and Rossi, 1980 and 1982; Garris *et al*, 1982, Luse, 1970, Mukai *et al*, 1980, Magarinos and McEwen 2000, Saravia *et al*, 2001; McEwen 2002). In addition, impaired long-term potentiation in the hippocampus (indicative of pre- and post-synaptic deficits) was reported (Biessels *et al*, 1996). The hippocampus is also vulnerable to damage by stroke and head trauma, susceptible to damage during aging, chronic stress (Sapolsky

1992), and sensitive to the effects of diabetes (Gispen and Biessels, 2000; Magarinos and McEwen 2000; Saravia *et al*, 2002). However, it is also a plastic and adaptable brain region that is capable of considerable structural reorganization. Studies from McEwen (2002) have shown that STZ induction perturbs structural plasticity of the hippocampus and its sensitivity to glucose and oxidative stress. His studies suggest that STZ-diabetes might cause a reduced number of dendrite spines and decreased total length of dendrites of pyramidal neurons of the hippocampus (Magarinos and McEwen, 1995). Other studies by Martinez-Tellez *et al* (2005) showed that dendritic morphological changes also occurs in pyramidal neurons located in structures related to cognitive processes such as, prefrontal cortex, occipital cortex and hippocampus, in the STZ-diabetic rats. The authors suggest that these results together with the available literature, indicate that nitric oxide (NO), GCs, stress, astrogliosis, and glutamate may participate in the dendritic morphological changes.

3.2. Behavior

Diabetes-related cognitive dysfunctions were first reported in 1922 (Miles and Root, 1922). Subsequent studies have demonstrated impairments in CNS function. Impairments in learning and memory, problem solving, and intellectual development have been documented in T1D patients (Ryan and Williams, 1993; Ryan *et al*, 1993; Kramer, 1998; Parisi and Uccioli, 2001; McCarthy *et al*, 2002; Schoenle, 2002). Cognitive dysfunction and impaired intellectual development are evident in a duration-related manner in T1D patients independent of hypoglycemic episodes (Kramer, 1998; Schoenle, 2002).

Stress and stress hormones affect different aspects of learning and memory. MR signaling can enhance performance on spatial hippocampal-dependent cognitive tasks (de Kloet *et al*, 1999) and its chronic blockade impairs spatial memory (Douma *et al*, 1998). Decreasing GR signaling attenuates the impairing effects of GR activation on cognition (Nicholas *et al*, 2006). Acute elevation of GCs facilitates the formation of memories of events associated with strong emotions (McGaugh, 2000; Roozendaal, 2000). Chronically, however elevated GC levels contribute to impairment of cognitive function and promote damage to brain structures such as the hippocampus (McEwen and Sapolsky, 1995; Lupien and McEwen, 1997; Sapolsky, 2002). This inverse-U function of GCs is a reflection of the diversity of receptors for GCs in the hippocampus (de kloet *et al*, 1993).

Impaired performances in the Morris water maze are typically observed in STZ-induced diabetes rats (Lowy *et al*, 1993; Biessels *et al*, 1996; Lupien and McEwen, 1997) and are associated with impaired LTP in the hippocampus (Biessels *et al*, 1996). It is reported that STZ diabetes does not disturb operant behaviors for food reward (Kaleeswari *et al*, 1986) but facilitates retention of passive avoidance in rats and mice (although not always) (Bellush and Rowland, 1989; Flood *et al*, 1990; Mayer *et al*, 1990). In addition, diabetic rodents consistently displayed performance deficits in more complex learning tasks, such as an active avoidance T-maze, or a Morris water maze

depending on the duration of STZ diabetes (Biessels *et al*, 1996 and 1998; Flood *et al*, 1990; Popovic *et al*, 2001). However, discrepancies exist among several behavioral studies and may be partially explained by differences in task complexity, animal models used and duration of diabetes. A key factor, however, appears to be the nature of the stimulus used in behavioral paradigms. There are clear indications that the physiological responses to a novel environment or to stressful stimuli, which are often part of learning paradigms, are larger in STZ-diabetic than in non-diabetic rodents (Bellush *et al*, 1991; Bellush and Rowland, 1989; Flood *et al*, 1990). For example, enhanced retention of simple passive avoidance task in diabetic rodents has been attributed to an increased sensitivity to the foot shock (Bellush and Rowland, 1989, Flood *et al*, 1990).

4. Scope of the thesis

4.1. Rational and Objective

Peripheral and autonomous neuropathies are well-known and devastating complications of type 1 diabetes. However, T1D can also impact the integrity of the CNS, and the reason why T1D affects CNS integrity remains to be elucidated.

Diabetic animals show high circulating glucocorticoid levels, increased sensitivity to stress, and morphological alteration in various brain areas. How these changes occur is not known, but hypercorticism *per se* can evoke a similar neurodegenerative cascade. The conditions of aberrant GCs levels appear (i) to enhance the vulnerability to metabolic insults of brain areas showing a high degree of plasticity, such as the hippocampus and (ii) may underlie the impairment of cognitive performance.

In T1D, a fundamental question in the central neuropathophysiology is whether GCs aggravate the functional and morphological signs of neurodegeneration and cognitive impairment. Therefore, the objective of the present study is to elucidate the role of GC excess and GC-stress system activity in T1D mice in relation to morphological indices for neuronal viability and cognitive performance.

4.2. Hypothesis

We hypothesize that under conditions of T1D, excess GCs and dysregulation of the GC-stress system will contribute to cerebral damage by making the brain more vulnerable to metabolic insults and causing concomitant cognitive disturbances. We propose that T1D leads to a more fragile state of the brain in which high levels of GCs may enhance the potential for damage and attenuate protective mechanisms, thus precipitating impairment in cognitive function. We expect to unravel GCs and diabetes interactions and relationships.

4.3. Questions to address

I. Is there neuronal damage in diabetes? (maybe as a consequence of GCs excess in diabetes?)
II. What is the pattern of HPA (re)activity in response to diabetes in time?
III. Do cognitive disturbances parallel these changes?
IV. Are GCs responsible for brain alterations in diabetes?
V. Will treatment with anti-glucocorticoids (RU486) prevent and/or restore these alterations?

4.4. Experimental approach

To allow generalization of the results, pharmacological and genetic animal models for diabetes, the STZ-induced and NOD mice, respectively, will be used. For both animal models there are indications of an aberrant functioning GC-stress system (i.e. GC hypersecretion). To test our hypothesis we planned:

I. To measure parameters of neuronal damage in NOD pre-diabetic, non-diabetic and diabetic mice and in STZ-diabetic and control mice.
II. To test HPA axis (re)activity in both models. For this purpose blood samples will be collected to measure basal concentrations of GCs and ACTH. Also central parameters of HPA activity (*in situ* hybridization of mRNA of MR, GR, CRH, AVP) will be measured.
III. To test cognitive abilities at certain time points of specific HPA (re)activity in the Morris water maze, novel-place recognition, open field, elevated plus maze, forced swimming test. General measures of activity will be recorded as well.
IV. To use the glucocorticoid antagonist RU486 in order to elucidate the role of GCs and attenuate vulnerability to damage and enhance protection. We discovered that RU486 acts like a double-edged sword: the antagonist blocks the damaging impact of excess GR stimulation while maintaining the beneficial effects of the 'neuroprotective' MR. After four consecutive days of RU486 administration, prevention and/or amelioration of neuropathological signs and restoration of cognitive abilities will be studied.

4.5. Chapters

Chapter 2 delineates the HPA axis functionality in a genetic model of T1D, the NOD mice. Central parameters of the HPA axis as well as C-peptide and cytokine levels were measured in pre-diabetes and diabetes states. In this model, the results suggest that an enhanced ACTH release may signal the onset of diabetes. **Chapter 3** addresses the underlying mechanism of hypercorticism in STZ mice. Central parameters of the HPA axis and specifically adrenal function were investigated at different time points after STZ-injection. The study demonstrates that adrenal hypersensitivity to ACTH precedes and

maintains the state of chronically elevated glucocorticoids levels. **Chapter 4** describes molecular parameters in the hippocampus of the STZ-diabetic mice that could reflect functional abnormalities of astrocytes and neurons. Parameters of astrocytic and neuronal disturbances such as apolipoprotein E and markers for oxidative stress and early gene expression respectively, were measured between control and diabetic mice. The results showed hippocampal disturbances in this model of T1D, which could be a primary basic mechanism underlying the well-known brain alterations associated with diabetes. **Chapter 5** reveals the role of GCs in STZ-induced diabetic mice, at the molecular and cognitive levels. After the administration of glucocorticoid receptor antagonist (RU486 or mifepristone) for 4 consecutive days to non-diabetic and diabetic mice, the novel placement recognition test was performed and molecular hippocampal parameters reflecting functional abnormalities were measured. Normalization of neuropathological signs and cognitive abilities was found. In **Chapter 6** the experimental data are discussed and placed in a conceptual framework highlighting the central action of glucocorticoids in the onset and progression of diabetes neuropathology.

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