Chapter 1

General introduction

I think animal testing is a terrible idea; they get all nervous and give the wrong answers.
- A Bit of Fry and Laurie
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Glucose homeostasis

Glucose levels in the blood are well-regulated. Glucose is a vital energy substrate, and it is therefore of paramount importance that its levels are controlled. High glucose levels cost much energy to maintain, and may lead to long-term complications such as microvascular and macrovascular damage. Low levels will impair energy availability, which is also undesirable – especially for the brain which is predominantly dependent on glucose for its energy. Ideal therefore would be a constant optimal level of glucose in the blood, which moreover can be adjusted in times with changed demand or supply.

The body has several mechanisms to ensure this homeostatic situation (5, 9). The supply of glucose to many tissues is regulated by the pancreatic hormone insulin. As a hydrophilic molecule, glucose needs to be transported across the cellular membrane to enter the cells. This is achieved by glucose transporter proteins, and the most sensitive of those is activated by insulin. Other tissues such as the brain have insulin-independent glucose transporters however, and can thereby always use glucose from the blood independent of the presence of insulin (3).

Because of its stimulation of glucose uptake, insulin is the key hormone to counteract high blood glucose levels. During hyperglycemia, insulin release from the pancreatic beta-cells is stimulated, and the increased insulin levels inhibit glucagon and stimulate uptake of glucose by the tissues that express the insulin-sensitive glucose transporter (especially muscle and fat) (5). The increased insulin levels also stop the release of glucose from the liver (7). This results in a reduction of blood glucose levels, and storage of energy. In addition, insulin is transported into the brain, where it is thought to increase satiety and thereby decrease food intake (43, 56).

In contrast, low blood glucose levels are counteracted by a range of hormones (5, 31, 34, 46, 57). The two major hyperglycemic hormones are glucagon and adrenaline. Glucagon is secreted from the pancreatic alpha-cells, and its main function is to stimulate glucose production and release by the liver. Adrenaline is released from the adrenal medulla and acts on most of the tissues in the body. Among others it increases glucose production by the liver and kidneys, inhibits glucose uptake by muscle and promotes lactate release, and stimulates glucagon secretion.

All these responses seem to be under control of the brain (26, 41, 51). The brain reacts to changes in blood glucose levels (2, 47), and thereby it can regulate the appropriate responses, such as stimulating insulin and inhibiting food intake when glucose levels are high, or stimulating glucagon and adrenaline when glucose levels are low (35, 42). The brain is therefore likely the major player in the control of glycemias.

Together these hormonal and neural mechanisms enable healthy organisms to maintain stable blood glucose levels, even in changing environments.
Diabetes and hypoglycemia

Diabetes is a chronic disease defined by impaired or absent insulin function. When untreated, this leads to a strongly reduced ability to counterregulate elevated blood glucose levels (hyperglycemia), a reduced ability to use glucose as energy source, and finally ketoacidosis. Hyperglycemia on the long term also leads to microvascular and macrovascular complications, increasing the risk for blindness, kidney failure, neuropathy, and cardiovascular disease (20). Hence, diabetes needs to be treated with therapies aiming to reduce blood glucose levels. Dietary changes and exercise may be sufficient to treat a mild diabetic state, but more pronounced diabetes needs to be treated with hypoglycemic drugs (45, 48). It may be clear that the precise treatment regimen of such drugs is important, because too high or improperly timed dosing may decrease blood glucose too much (hypoglycemia).

Drug-induced hypoglycemia is particularly riskful for several reasons. First, it may come unanticipated. Second, such drugs’ action often lasts for many hours. Third, the drug’s effect may occur simultaneously with other factors decreasing glucose levels, such as fasting or physical activity. Finally, diabetes is accompanied by impairment of the normal counterregulatory responses to hypoglycemia (12, 37) – as discussed later. Therefore, while significant hypoglycemia is very rare in healthy persons, it is common for diabetes patients, especially those on insulin therapy.

Hypoglycemia poses a considerable problem for the treatment of diabetes. Although its mortality is low (hypoglycemia only rarely results in severe injury or death), it is a profound psychosocial problem, with strong effects on morbidity and quality of life (22, 23). All diabetes patients on insulin therapy fear hypoglycemia; afraid of getting into severe hypoglycemia, being unable to perform normal daily functions, or even losing consciousness. Many patients worry as much about severe hypoglycemia as they do about the serious long-term complications of diabetes (44). This fear of the incapacitating effect of hypoglycemia results in a non-optimal treatment of diabetes, because it causes many physicians and patients to shun the insulin doses needed to fully normalize blood glucose levels. This results in chronic hyperglycemia which significantly increases the risk for the long-term microvascular and macrovascular complications – increasing morbidity and mortality.

This reduced compliance is particularly a problem in intensive insulin therapy, which aims specifically at avoiding those long-term complications. Intensive therapy allows a much tighter glucose control, bringing the daily glucose profile closer to a normal profile. As shown in the Diabetes Control and Complications Trial (DCCT) (20, 38, 40), intensive therapy greatly reduces the incidence of the aforementioned long-term microvascular and macrovascular complications of diabetes. It does however increase the incidence of hypoglycemia (19, 21), because it is very difficult to regulate glucose levels close to normal without an increased risk of hypoglycemia – which patients and their physicians will try to avoid, at the cost of a suboptimal glucose profile (52).

Taken together, it becomes clear that hypoglycemia is the main limitation in the treatment of diabetes (14, 18, 25). In addition, the problem of hypoglycemia is becoming
pertinent for many more patients, because the number of diabetes patients on intensive insulin therapy has greatly increased since the DCCT. It is therefore very important to understand the physiological mechanisms by which hypoglycemia is detected and counteracted.

**Counterregulation to hypoglycemia**

As mentioned before, low glucose levels are counterregulated by a variety of responses, some of which may be impaired in diabetes.

In healthy humans or animals, a number of systems respond to hypoglycemia. A hierarchy for these counterregulatory responses has been described (13, 28, 39). Generally, the first response to a decrease in blood glucose levels is the suppression of endogenous insulin production, which happens already after a small decline in blood glucose. When glucose levels drop further, secretion of the most important hyperglycemic hormones starts – first glucagon, followed by adrenaline, while corticosteroids and growth hormone also may respond (5, 33, 55). Low glucose levels will in addition generate a feeling of hunger, and food intake is hence the normal behavioral response to hypoglycemia.

These responses are mediated by the autonomic nervous system (30, 31). When glucose levels keep going down, sympathetic activity increases further, and together with the concomitant adrenaline response, this produces the sensations that diabetes patients learn to associate with hypoglycemia (such as sweating and trembling (6, 10)). These sensations are very important, since they signal the upcoming hypoglycemia – enabling the subject to become aware of the situation and take appropriate action (intake of carbohydrates) and perhaps also adjust therapy regimen to avoid repetition of the problem.

In diabetes, the counterregulatory responses to hypoglycemia are of even greater importance. Not only do diabetes patients experience many hypoglycemic episodes, but those episodes are also of a serious and longer-lasting nature. Unfortunately, diabetes is accompanied by several defects in the normal counterregulatory responses (12). Firstly, the pancreatic beta-cells are malfunctioning, and therefore the important early counterregulatory response to hypoglycemia – inhibition of insulin secretion – is partially or completely impaired. Severe hypoglycemia is indeed less common in diabetes patients who still have some residual beta-cell functionality (as shown by the presence of C-peptide in the blood) (21). Furthermore, the glucagon response to hypoglycemia disappears during the first years after the onset of diabetes (37). This means that two of the most important counterregulatory responses to hypoglycemia are impaired in diabetes patients. A proper adrenaline response and an accurate awareness of hypoglycemia are therefore more than crucial for the diabetes patient to recover from a hypoglycemic episode.

It is unknown how the different counterregulatory responses are coordinated in relation to each other. The brain is involved in the control of energy metabolism (36, 49, 50) and is known to be involved in the counterregulatory responses to hypoglycemia (4, 47), but detailed information on the areas involved and the neurochemical background of this coordination is scarce. It is also unknown whether the counterregulatory responses adapt to situations with higher energy demand or lower energy availability (such as during exercise.
or fasting, situations where diabetes patients have a greatly increased risk of hypoglycemia). Studies suggest that counterregulatory responsivity may increase during fasting (32, 53), but this has not been experimentally studied yet for insulin-induced hypoglycemia. Thus more investigations into the mechanistic nature of hypoglycemia counterregulation are needed.

Recurrent hypoglycemia and hypoglycemia unawareness

As was concluded in the previous paragraphs, hypoglycemia occurs more often in diabetes patients, the hypoglycemic episodes are more severe and longer-lasting, and essential counterregulatory responses are impaired (12, 37). There is an other negative effect: the more often hypoglycemia occurs, the greater the risk that it happens again (15). An increased frequency of hypoglycemia impairs the counterregulatory responses to hypoglycemia, a phenomenon known as Hypoglycemia-Associated Autonomic Failure (HAAF) (1, 12, 16). HAAF affects most major counterregulatory responses to hypoglycemia, including adrenaline, meaning that a stronger reduction of blood glucose levels is needed before this important response is triggered. In addition, the feelings associated with hypoglycemia (such as sweating, trembling, increased pulse) are reduced as well, a condition called hypoglycemia unawareness (5, 8, 29).

Taken together, patients with HAAF and hypoglycemia unawareness fail to produce the appropriate autonomic counterregulatory responses to counteract hypoglycemia, while at the same time they are less aware of their becoming hypoglycemic. The consequence is that hypoglycemia occurs more and more often, which again further worsens the above mentioned defects – a vicious circle which can only be broken by scrupulously avoiding hypoglycemia (11, 17, 54). It is estimated that 20-25% of insulin-treated patients have impaired hypoglycemia awareness, while this increases to around 50% for patients who have been on insulin therapy for 25-30 years (24, 27, 44).

We may therefore conclude that HAAF, the impairment of the autonomic responses to hypoglycemia, is a serious problem associated with intensive insulin therapy. Hypoglycemia in itself is not necessarily dangerous, as long as the patient is aware of the declining blood glucose levels and can take appropriate action (intake of carbohydrates, and avoiding potential dangerous activities such as driving a vehicle); therefore preservation of hypoglycemia awareness is important for every patient on insulin therapy. Intensive insulin therapy has many advantages – and many patients have switched to this therapy form. This increases the occurrence of hypoglycemia unawareness, and it is therefore crucial to understand the problem of recurrent hypoglycemia. However, the underlying mechanisms that cause the development of HAAF and hypoglycemia unawareness is still unclear.

Counterregulation to acute and recurrent hypoglycemia in rats

From the previous paragraphs it is clear that we need to obtain more insight in 1) the underlying mechanisms that control the counterregulatory responses to insulin-induced hypoglycemia, and the factors that may influence this regulation such as nutritional state; 2)
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the neurochemical nature and the central nervous pathways controlling these responses; and
3) the development of defects due to recurrent hypoglycemia.

The studies described in this thesis are aimed at investigating these three research
questions. Due to the fundamental nature of these questions as well as to enable brain
investigations, all studies were performed in rats.

The first section of this thesis investigates the regulation and adaptation of the
counterregulatory responses to insulin-induced hypoglycemia. We studied these responses
by administering different doses of insulin in fed and fasted rats (Chapter 2). Insulin was
infused intravenously and the counterregulatory responses were determined in blood
samples taken throughout the study period. This study also addressed the question how a
change in nutritional state (i.e. fasting) may affect the counterregulation to hypoglycemia. In
the next study the effect of another change in energy availability was examined. Here we
studied counterregulatory responses when insulin-induced hypoglycemia was combined
with reduced availability of energy from fatty acids (Chapter 3).

The second section of this thesis focuses on the brain mechanisms involved in the
counterregulation to hypoglycemia. Data from the literature pointed to a possible role for
noradrenergic neurotransmission in the hypothalamus in the activation of the
counterregulatory responses. To investigate this, noradrenaline release in the hypothalamus
during euglycemia and hypoglycemia was measured by means of in vivo microdialysis
(Chapter 4), while in another study noradrenergic neurotransmission in the hypothalamus
during hypoglycemia was pharmacologically blocked by adrenoceptor antagonists (Chapter
5).

The last section of this thesis addresses the issue of recurrent hypoglycemia. Since no
established animal model existed for the development of Hypoglycemia-Associated
Autonomic Failure, we performed a number of pilot studies to investigate the development
of HAAF in rats, and to characterize some of the associated phenomena, such as the
individual differences in susceptibility to HAAF and the importance of the hypoglycemic
drug used (Chapter 6).

References

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Section I – Counterregulation, insulin, and the nutritional state

Westheimer’s Discovery:
A couple of months in the laboratory can frequently save a couple of hours in the library.