

Severe hypoglycaemia in type 1 diabetes: impact of the renin-angiotensin system and other risk factors

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This review has been accepted as a thesis together with eight previously published papers by the University of Aarhus, April 6, 2008, and defended on September 25, 2009.

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Dan Med Bull 2009;56:193-207

INTRODUCTION

Hypoglycaemia is an unavoidable side effect to insulin therapy of diabetes. In daily life some hypoglycaemic episodes are recognised by the patients [1] and corrected by ingestion of glucose. Others, however, remain unrecognised [1] and are either corrected by a pre-planned meal or snack, by waning of the insulin effect, and/or by counterregulatory carbohydrate mobilisation. Occasionally, unrecognised episodes progress into severe hypoglycaemia with cognitive impairment and the need for assistance from other persons in order to manage the situation. Such episodes represent the most feared side effect to insulin treatment [2, 3] and are regarded as the major limiting factor for achievement of recommended glycaemic targets in type 1 diabetes [4]. While prevention and treatment of late diabetic complications is the clinical and scientific focus of most diabetologists, the patients are just as concerned about the daily risk of severe hypoglycaemia as they are about the future risk of suffering from invalidating late complications such as blindness and kidney failure [2]. Patients with recurrent severe hypoglycaemia often report depressed mood and poor quality of life [5] and fear of hypoglycaemia may interfere with daily living and preclude patients from participation in social activities [6]. The concern about living with

risk of severe hypoglycaemia is not limited to the patients themselves but shared by their close relatives. Severe hypoglycaemia is, in fact, the most significant determinant of spouses' involvement in their partners' disease [7].

Therefore, prevention of severe hypoglycaemia is an important issue in diabetes care that may contribute to improvement of outcome measures both in terms of hard endpoints such as glycaemic control and late complications [8] and more soft measures such as quality of life for a large number of people with type 1 diabetes.

The studies that constitute this thesis were conducted to assess the significance of severe hypoglycaemia as a clinical problem in the type 1 diabetic population. Specifically, we aimed to assess the frequency of severe hypoglycaemia after the implementation of intensive basal-bolus insulin therapy as the standard treatment resulting from the outcome of the Diabetes Control and Complications Trial (DCCT) [8] and to evaluate the impact of known risk factors on occurrence of severe hypoglycaemia. Finally, we sought to identify new markers that could contribute to improved prediction and inspire to novel preventive measures, of severe hypoglycaemia.

METHODOLOGICAL CONSIDERATIONS

SEVERE HYPOGLYCAEMIA

Literally, the term hypoglycaemia refers to an abnormally low concentration of glucose in the blood. As such the definition covers a broad range of clinical conditions ranging from asymptomatic episodes (unrecognised or silent hypoglycaemia) to episodes with severe brain dysfunction, coma and fits (Table 1). In practise there is no uniformly agreed definition of severe hypoglycaemia and it thus varies between studies. The term is most often reserved for episodes with such a degree of cognitive impairment that the patient needs assistance from another person in order to attain normoglycaemia [2, 9-11]. This definition – which was recently recommended by the Workgroup on Hypoglycemia, American Diabetes Association [12] – is justified by the fact that these episodes represent situations where patients are at danger of suffering accidents due to impaired ability to take care of themselves. Some studies, however, define severe hypoglycaemia solely by the requirement of parenteral treatment in terms of intravenous glucose or intramuscular glucagon [13] or by the need for admission to hospital [14].

In order to prove that an episode with cognitive impairment is due to hypoglycaemia, and not to some alternative cause, episodes can be validated according to Whipple's triad that consists of three criteria: I) characteristic hypoglycaemic symptoms; II) biochemical confirmation; III) adequate response to treatment with glucose or glucagon. According to Whipple's triad only episodes fulfilling all criteria can be considered definite, whereas those fulfilling two criteria can only be considered probable. Unfortunately, Whipple's

Table 1. Classification of hypoglycaemic episodes.

<i>Biochemical classification</i> Biochemical hypoglycaemia	Episodes with blood, plasma, or interstitial glucose concentration below a defined limit
<i>Clinical classification</i> Silent (asymptomatic) hypoglycaemia	Episodes with biochemical hypoglycaemia not recognised by patient or surroundings (Glucose measurement mandatory)
Mild (symptomatic) hypoglycaemia	Episodes with presumed hypoglycaemic symptoms recognised and self-treated without assistance from other persons (Glucose measurement not mandatory)
Severe hypoglycaemia	Cognitive impairment requiring assistance from other persons to restore glycaemic level (Glucose measurement not mandatory)
Hypoglycaemic coma	Severe cognitive impairment with coma or fits and/or requiring parenteral treatment (IV glucose or IM glucagon) (Glucose measurement not mandatory)

triad is not widely used in clinical studies and the validity of reported events is consequently variable.

Severe hypoglycaemia can be assessed retrospectively based on patients' recall of episodes in a defined period, or recorded prospectively. We assessed the recall of severe hypoglycaemia in a cohort of unselected patients with type 1 diabetes by comparing prospectively recorded number of episodes during a one-year period with recall at the end of the period [15]. The study showed that recall of severe hypoglycaemia is well-preserved within a one-year period. Carefully collected retrospective data on severe hypoglycaemia may therefore be regarded with confidence. Prospectively recorded episodes are in general of high validity. Thus, in our prospective study 50% of the episodes could be classified as definite according to Whipple's triad [16]. The remaining episodes were classified as probable because, in practice, many episodes are treated as hypoglycaemia without any glucose measurement as patients and their relatives are familiar with the symptoms. There was no significant reporting of non-hypoglycaemic events. The vast majority of studies of severe hypoglycaemia in type 1 diabetes rely on retrospective data [2, 9-11, 17]. Only four prospective observational studies have been published [15, 16, 18, 19].

Close relatives could potentially provide data on severe hypoglycaemia. We compared spouse reporting with patient reporting in a cross-sectional survey and found that the spouses estimated significantly (1.7-fold) higher rates of severe hypoglycaemia than the patients did and that there was some agreement between patients and spouses with a kappa value of 0.40 [7]. This is in agreement with an earlier study that reported a kappa value of 0.48 [20]. It is not known whether the numbers reported by the spouses or by the patients themselves are more valid. The spouse-based approach is obviously limited by the facts that spouses do not witness all events and that assessments can only be done for patients living together with a partner.

ASSESSMENT OF HYPOGLYCAEMIA AWARENESS

Hypoglycaemia awareness indicates the ability of the patient to detect impending or existing hypoglycaemic episodes. If this ability is reduced or lost the resulting conditions are termed impaired awareness and hypoglycaemia unawareness, respectively [21, 22]. Hypoglycaemia awareness is a complex and incompletely understood phenomenon. Normal awareness is dependent on perception of autonomic symptoms [23] and as these are often blunted along the course of diabetes as a result of blunted adrenaline responses and hypoglycaemia associated autonomic failure [22] many patients will experience permanently reduced awareness during their life with diabetes. A reversible impairment of awareness, probably tachyphylactic of nature, has been described as result of tight glycaemic control with exposure to recurrent hypoglycaemic episodes, mostly in the early stages of type 1 diabetes [24, 25].

Due to the complexity of hypoglycaemia awareness, there is, at present, no general agreement on a classification. The clinical assessment of awareness can be based on four different types of data: I) subjects' own estimation of their state of awareness; II) self-monitored glucose data or continuous glucose monitoring data with scoring of symptoms in case of hypoglycaemia; III) an objective measure in terms of the response to a provoked hypoglycaemic challenge; or IV) an evaluation of the clinical consequence of impaired awareness: occurrence of severe hypoglycaemia; or a combination of these methods. A simple method for classification is necessary in daily clinical practice and in population studies. This disqualifies objective assessments based on experimentally induced hypoglycaemia. Standardised self-monitored glucose data are difficult to obtain in the general population with type 1 diabetes and a classification based on such data may be heavily influenced by individual self-monitoring patterns. In prospective studies evaluating risk factors for severe hypoglycaemia, the classification of hypoglycaemic awareness should not include prior incidence of severe hypoglycaemia, because, per se, this is a strong, yet grossly unexplained, risk

factor for future severe hypoglycaemia that is also influenced by other risk factors. This leaves the patients' own assessment as the most appropriate data source. Such an estimate can be based on a general screening question or on more elaborate questionnaires taking into account different aspects of awareness.

Two methods for assessment of hypoglycaemia awareness have previously been reported and tested in prospective studies. The Clarke method [19] is an elaborate score derived from the answers to eight questions about hypoglycaemia and hypoglycaemic symptoms. The use of this method in retrospective studies is hampered by the inclusion of number of episodes of severe hypoglycaemia in the preceding year in the score. The Edinburgh scale scores awareness on a seven-point Likert scale according to the simple screening question "Do you know when your hypos are commencing?" [18]. Both the Clarke and the Edinburgh methods are dichotomously classifying subjects as having normal or impaired awareness. The Clarke method, however, leaves a minor intermediate group unclassifiable. We assessed awareness by a number of questions and evaluated their impact on rate of severe hypoglycaemia [15]. The pertinent question was: "Do you recognise symptoms, when you have a hypo?". This question is equivalent to questions used by other groups, alone or as part of more elaborate scoring systems [18, 19]. The categories for answers were: "always", "usually", "occasionally", "never", and "don't know". Subjects scoring "always" were classified as having normal awareness, those reporting "usually" as having impaired awareness, and those answering "occasionally" or "never" as having severely impaired awareness (hypoglycaemia unawareness). This method, in contrast to the previously published methods, introduced an intermediate group to encompass the clinical impression that hypoglycaemia awareness is not an all-or-none phenomenon but rather a sliding scale [21]. In addition, patients scored their usual hypoglycaemic warning symptoms on a scheme as introduced and validated by the Edinburgh group [26]. The summed scores for autonomic and neuroglycopenic symptoms were calculated and the symptom category with the highest score was defined as the dominating symptom category. Finally, the subjects were asked to estimate their usual blood glucose threshold for occurrence of hypoglycaemic symptoms. The study showed that using the simple general screening question resulted in the best discrimination between aware and unaware patients in terms of risk of severe hypoglycaemia [15]. Adding information from the specific questions did not improve the performance of the classification system [15]. The significance of the classification was fully comparable to the Clarke and the Edinburgh methods [18, 19] and the introduction of an intermediate group even seemed to improve the discrimination between aware and unaware subjects in terms of rate of severe hypoglycaemia compared to the other methods. Furthermore, the method performed similarly in different populations across language barriers [27] and was reproducible in serial assessments [15].

A recent comparative study published by The Edinburgh Group challenges the performance of our method and concludes that it is too simplified and overestimates the proportion with impaired awareness [28]. The paper and the conclusions herein are, however, grossly flawed by misquotation of our method including a failure to acknowledge and incorporate into the analysis that our method operates with three categories in contrast to the other methods [29]. Preliminary data from a comparative study from our group suggests that our method identifies a proportion of subjects with severely impaired awareness, which is comparable to the groups classified as having impaired awareness by the other methods [30]. In addition, our method stratifies those with normal awareness according to the other methods in two groups; one with normal awareness and very low rate of severe hypoglycaemia and one with impaired awareness and intermediate rate [30]. The resulting discrimination between the two extremes by our method is better than that of the other methods, which is to be expected due to the inclusion of an intermediate category.

We subsequently posed the same question to the spouses. This re-

Table 2. Incidence and prevalence of severe hypoglycaemia. Shown are non-interventive studies sorted according to definition of severe hypoglycaemia and – for comparison – the DCCT study.

Reference	Design	Definition of SH	Incidence ep./pt.yr.	Prevalence per year	Comment
Goldgewicht 1983 [17]	Retrospective survey	Third party assistance	N.a.	26%	
Hepburn 1990 [35]	Retrospective survey	Third party assistance	N.a.	31%	
Pramming 1991 [2]	Pro- and retrospective	Third party assistance	1.4 1.5	N.a.	
MacLeod 1993 [9]	Retrospective survey	Third party assistance	1.7	30%	
Gold 1994 [18]	Prospective Observational	Third party assistance	0.5-2.8 mean 1.7	66%	Selected: +/- aware
ter Braak 2000 [11]	Retrospective survey	Third party assistance	1.5	41%	
Pedersen-Bjergaard 2001 [10]	Retrospective survey	Third party assistance	1.1	N.a.	
Pedersen-Bjergaard 2003 [15]	Prospective Observational	Third party assistance	1.0	35%	
Pedersen-Bjergaard 2004 [27]	Retrospective survey	Third party assistance	1.3	37%	
Donnelly 2005 [32]	Prospective Observational	Third party assistance	1.2	N.a.	
UK Study Group 2007 [33]	Prospective Observational	Third party assistance	1.1-3.2 mean 2.2	22-46% 35%	Selected: +/- long duration
Mühlhauser 1991 [42]	Prospective Observational	Parenteral Treatment	0.25	N.a.	Selected: +/- nephropathy
Clarke 1995 [19]	Prospective Observational	Coma or parenteral	0.9-2.6 mean 1.8	N.a.	Selected: +/- aware
Mühlhauser 1998 [13]	Prospective Observational	Glucose IV or glucagon	0.2	N.a.	
Pedersen-Bjergaard 2004 [27]	Retrospective survey	Coma, glucose IV or glucagon	0.4	N.a.	
Leese 2003 [14]	Register study	Emergency treatment	0.1	7%	
Hirai 2007 [34]	Retrospective survey	Coma or hospitalisation	N.a.	14%	
DCCT 1997 [31]	Prospective Intervention	Third party assistance	0.2-0.6 mean 0.4	11-27%	Selected

sulted in some agreement between patients and spouses in classification of awareness with a kappa value of 0.44 [7], which was higher than the value of 0.20 obtained in a study using a similar broad screening question [20].

INCIDENCE AND PREVALENCE OF SEVERE HYPOGLYCAEMIA

Prior to the DCCT [8], when the standard insulin regimen was one- or two-dose treatment the incidence of severe hypoglycaemia requiring assistance for treatment in patients with type 1 diabetes was reported to be well above one episode per patient-year (Table 2) [2, 9]. In the DCCT the incidence was reportedly much lower, both in the conventionally treated group (0.2 episode per patient-year) and in the intensively treated group (0.6 episode per patient-year) despite the fact that the glycaemic control was much tighter than in the earlier studies [31]. We addressed this controversy in a cross-sectional survey in a cohort of 1076 consecutive patients in two Danish and two British clinics. The incidence and yearly prevalence of severe hypoglycaemia was 1.3 episode per patient-year and 37%, respectively [27]. These numbers are similar to the data from the pre-DCCT era [2, 9] and are confirmed prospectively in our one-year study of 213 consecutive type 1 diabetic patients [16] and by studies from The Netherlands [11] and UK [32, 33] on cohorts treated with state-of-the-art basal-bolus regimens (Table 2). The reported incidence and prevalence of severe hypoglycaemia with coma or requiring intramuscular glucagon or intravenous glucose administration are 0.1 to 0.4 episodes per patient/year [13, 14, 27] and 7% to 14% [14, 34], respectively.

Our four-clinic survey [27] furthermore showed a pronounced skewness in the distribution of severe hypoglycaemia (Figure 1)

with 5% of subjects accounting for 50% of the episodes, as confirmed by our prospective study (Figure 1) [15]. This finding is in accordance with the experience from daily clinical practice.

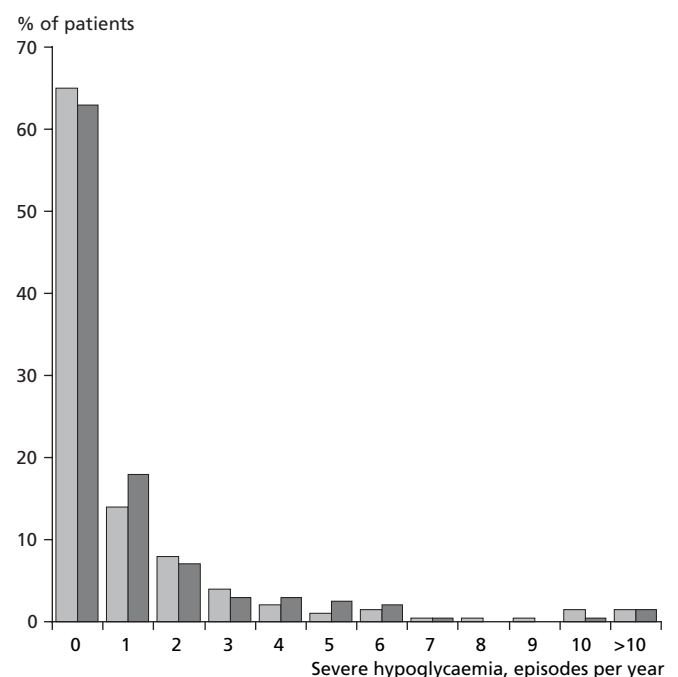


Figure 1. Distribution of episodes with severe hypoglycaemia assessed retrospectively (light bars; n=1076) [27] and prospectively (dark bars; n=230) [15].

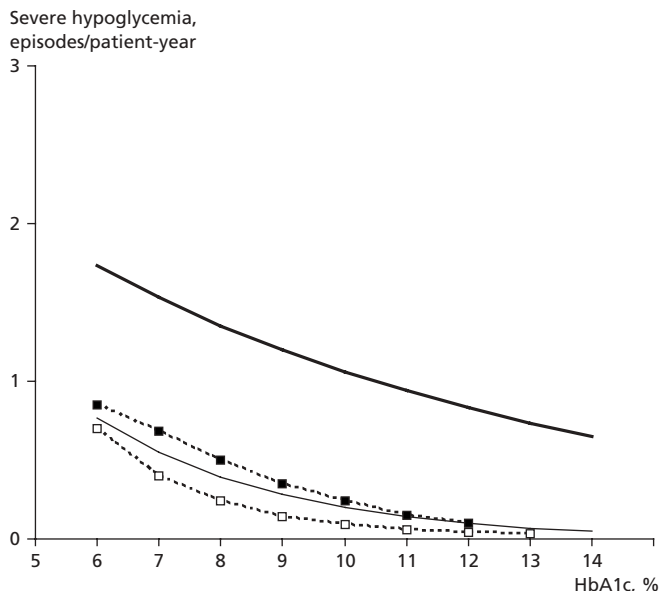


Figure 2. Univariate relationship between risk of severe hypoglycaemia and HbA1c. Shown are data on 1076 consecutive patients (thick line) and 209 patients selected by criteria to mimic the characteristics of the DCCT cohort (thin line) [27]. For comparison, corresponding data from the DCCT [31] are inserted (dotted lines; ■: intensively treated group, □: conventionally treated group).

Compared with the general population of patients attending diabetes clinics [11, 27] the subjects included in the DCCT were much younger with shorter duration of diabetes and fewer late complications. Furthermore, recurrent severe hypoglycaemia was an exclusion criterion in that study [8]. In order to assess whether the lower incidence reported by the DCCT is representative for the general type 1 diabetic population or explained by differences in cohort characteristics, we selected the subgroup of patients from our four-clinic survey that fulfilled the DCCT inclusion criteria. In this subgroup the incidence of severe hypoglycaemia was 0.4 episodes per patient-year, i.e. between the incidences of the two DCCT groups

(Figure 2) [27], supporting that the lower rates of severe hypoglycaemia in the DCCT are primarily explained by the shorter duration of diabetes and the exclusion of patients with recurrent severe hypoglycaemia.

The introduction of basal-bolus insulin regimens as standard in the treatment of type 1 diabetes has not affected the risk of severe hypoglycaemia which today is just as important a clinical problem as in the pre-DCCT era.

THE INFLUENCE OF RISK FACTORS

The skewed distribution of severe hypoglycaemia (Figure 1) suggests a clinically significant impact of risk factors on individual susceptibility to severe hypoglycaemia. Such factors may be related to diabetes itself, its complications, its treatment or to lifestyle and other psychosocial issues, and may affect the risk of severe hypoglycaemia by different mechanisms.

DIABETES-RELATED RISK FACTORS

Hypoglycaemia awareness

Normal hypoglycaemia awareness is important for the recognition of hypoglycaemic warning symptoms and consequently for taking appropriate countermeasures during hypoglycaemic episodes. Impaired awareness is prevalent (25-58%) depending on cohort and definition [11, 13, 15, 16, 27, 35] and the prevalence increases with long duration of diabetes [2, 27]. Tight metabolic control may reduce hypoglycaemia awareness [36] due to frequent exposure to hypoglycaemia [37, 38], an effect that has been reported to be at least partially reversible upon avoidance of hypoglycaemia [24, 25]. Impaired awareness is the most significant risk factor for severe hypoglycaemia in most previous studies (Table 3) with two- to six-fold increased rates among subjects with hypoglycaemia unawareness [18, 19]. In accordance, in both our retrospective [27] and prospective studies [16] impaired hypoglycaemia awareness and unawareness were associated with a clinically important increased rate of severe hypoglycaemia (six-fold and 10-21-fold, respectively).

Residual beta-cell function

The loss of endogenous insulin production in type 1 diabetes result-

Table 3. Risk factors for severe hypoglycaemia as reported in the literature.

Reference	Awareness	HbA1c	Duration of DM	Others	Comment
Hepburn 1990 [35]	+ (Edinburgh)	N.a.	N.a.	N.a.	
Pramming 1991 [2]	N.a.	+	-		Episodes with unconsciousness only
Mühlhauser 1991 [42]	N.a.	+	-	Low BMI, peripheral neuropathy	Pts. with elevated creatinine only
MacLeod 1993 [9]	+ (Edinburgh)	-	-	Previous severe hypoglycaemia	
Gold 1994 [18]	+ (Edinburgh)	N.a.	N.a.	N.a.	
Clarke 1995 [19]	+ (Clarke)	N.a.	N.a.	N.a.	
DCCT 1997 [31]	N.a.	+	-	Previous severe hypoglycaemia, C-peptide, female gender, adolescence	Female gender only in conventional group
Mühlhauser 1998 [13]	- (own score)	-	-	C-peptide, previous severe hypoglycaemia, low social class	
ter Braak 2000 [11]	+ (Clarke)	-	-	Neuropathy, beta-blockers, alcohol, smoking	
Allen 2001 [41]	N.a.	+	N.a.	Higher age	
Pedersen-Bjergaard 2001 [10]	+ (Hillerød)	-	+	C-peptide, ACE activity	
Leese 2003 [14]	N.a.	-	+	Higher age, poor socioeconomic status	
Pedersen-Bjergaard 2003 [16]	+ (Hillerød)	-	-	ACE activity	
Pedersen-Bjergaard 2004 [27]	+ (Hillerød)	-	-	Smoking, peripheral neuropathy	
Hirai [34]	N.a.	-	-	Smoking, waist-hip ratio	

ing from the progressive beta-cell destruction is followed by impairment of the glucagon response to hypoglycaemia. This is probably explained by the fact that rapidly decreasing intra-islet insulin concentrations, which is a key feature in impending hypoglycaemia in non-diabetic persons, is a major trigger of glucagon response [39]. This mechanism is obviously compromised with loss of beta-cell function. In clinical practice, endogenous insulin production can be evaluated by C-peptide concentration measured either at random with simultaneous plasma glucose measurement or following glucagon or standardized meal stimulation. A diminished glucagon response to hypoglycaemia has been demonstrated in C-peptide negative patients [40]. In a study defining C-peptide negativity as a random concentration <100 pmol/l and using multiple regression analysis, C-peptide deficient subjects had a hazard ratio of 4.0 for reporting severe hypoglycaemia [13]. In our retrospective study, C-peptide negativity (random concentration <10 pmol/l) was significantly associated with increased rate of severe hypoglycaemia in uni- and multivariate analyses with a relative rate of 1.8 [10]. In the prospective study, the association disappeared in the multiple regression analyses indicating that there was no independent effect of C-peptide status in that study [16]. The disagreement between our studies [10, 16] and the German study [13] may be explained by differences in cut-off value for C-peptide negativity or by differences in the variables included in the multivariate models.

Duration of diabetes

Both hypoglycaemia awareness [2, 27], endogenous insulin production – and thereby glucagon response [22] – and catecholamine responses to hypoglycaemia decline with duration of diabetes [22], and long duration has been associated with increased risk of severe hypoglycaemia. In our studies, long duration of diabetes was significantly associated with high rate of severe hypoglycaemia in univariate analyses (relative rate 1.4-1.5 per ten years) but the effect disappeared in the multiple regression analyses showing that long duration per se is not a risk factor [10, 16, 27]. This is in accordance with the majority of other studies (Table 3).

Level of glycaemic control

Low HbA1c was reported as a risk factor for severe hypoglycaemia in several earlier studies [2, 42]. Also in the DCCT [31] and in another cohort of young patients with short duration of disease [41] HbA1c was an important risk factor for severe hypoglycaemia (Table 3). This finding has not been reproduced in more recent studies with cohorts of unselected patients [11, 13], including our own [10, 16, 27]. In our multicentre survey, we evaluated the association between HbA1c and rate of severe hypoglycaemia in the subgroup fulfilling the inclusion criteria of the DCCT and found an effect resembling that of the DCCT with a relative rate of 0.7 per 1% absolute increment in HbA1c (Figure 2) [27]. This suggests that the importance of HbA1c is more pronounced in younger subjects with less complicated diabetes than in those attending most clinics or, perhaps more likely, that the level of glycaemic control in patients with long-standing diabetes is primarily determined by their individual risk of severe hypoglycaemia.

Other diabetes-related risk factors

In accordance with most studies [11, 13], age was not an independent risk factor for severe hypoglycaemia in our studies when duration of diabetes was accounted for [10, 16, 27].

The risk of severe hypoglycaemia was similar in the two sexes [27] although it tended to be higher in females. This is in accordance with the DCCT that reported female gender as a risk factor for severe hypoglycaemia [31]. However, this finding has not been reproduced in other studies [10,11].

The presence of late diabetic complications has been associated with severe hypoglycaemia in some studies. Increased risk of severe hypoglycaemia has been reported in subjects with nephropathy

[42]. This was not reproduced in our four-clinic survey, which, however, did not include patients with end stage renal failure [27]. In this study, peripheral neuropathy was an independent predictor of severe hypoglycaemia, a finding that has been reported for episodes with coma in another study [11]. Interestingly, in the subgroup selected to mimic the characteristics of the DCCT cohort in our four-clinic survey, presence of retinopathy was associated with severe hypoglycaemia [27]. This shows that the relationship between late diabetic complications and severe hypoglycaemia is sensitive to selection and this may contribute to the explanation of the poor concordance between studies on this subject.

In our four-clinic survey there was no association between the mode of insulin treatment and risk of severe hypoglycaemia [27]. At the time of the survey the vast majority of patients were on basal-bolus therapy with regular human insulin and NPH insulin. Thus, an impact on the rate of severe hypoglycaemia of rapid- and long-acting analogues could not be assessed. Open-labelled clinical trials have suggested a trend towards a reduced risk of severe hypoglycaemia by these agents, but none of the studies have been powered to detect clinically relevant differences in rate of severe hypoglycaemia [43, 44]. This is partially explained by exclusion of high-risk patients from these studies resulting in very low rates of severe hypoglycaemia. There is only one blinded study – with negative result [45] – and only one trial in high-risk (hypoglycaemia unaware) patients suggesting a beneficial effect of lispro on rate of severe hypoglycaemia, which failed to reach statistical significance [46]. A detailed evaluation of the effect of insulin analogues on rates of severe hypoglycaemia is beyond the scope of this thesis. Compliance with insulin treatment is an important problem as erroneous administration of insulin (e.g. repetition of already taken dose) is often reported in cases of severe hypoglycaemia [47]. The quantitative impact of such treatment errors on risk for severe hypoglycaemia is, however, not known.

LIFESTYLE-RELATED RISK FACTORS

Our multicentre survey [27] showed that subjects, who were married or living with a partner, reported a two-fold higher incidence of severe hypoglycaemia compared to those living alone. Rather than being due to a real effect of partnership this might be explained by spouses' influence on reporting. This is supported by the finding from our spouse survey of a 1.7 times higher rate of severe hypoglycaemia reported by spouses than by the patients themselves [7].

Compliance with meal ingestion is regarded as important to avoid severe hypoglycaemia. In accordance, omission of snacks or meals is a frequently reported cause of severe hypoglycaemia in case series [47, 48]. Physical exercise has been associated with severe hypoglycaemia in case series [49]. We were not able to establish a relationship between frequency of exercise bouts (defined by at least 30 minutes of rapid walking intensity) and risk of severe hypoglycaemia in our four-clinic survey [27]. This may be due to the method of assessment, to subjects frequently training being better to take countermeasures against exercise and post-exercise hypoglycaemia, or to the fact that most often exercise bouts associated with hypoglycaemia are unforeseen.

Use of tobacco

Smoking was identified as a novel independent risk factor for severe hypoglycaemia in our survey [27]. The rate of severe hypoglycaemia was particularly elevated in smokers who also used alcohol (relative rate 2.1 compared to non-drinking non-smokers). The association between smoking and severe hypoglycaemia is supported by two other studies [11, 34] but contradicted by one study [13]. Our finding may have multiple explanations. Firstly, smoking affects overall carbohydrate metabolism. A recent study in non-diabetic subjects reported impaired muscle glycogen recovery following exercise in smokers [50] and a similar phenomenon may be present following

Table 4. Identification of psychoactive drugs and alcohol in blood screens obtained in 141 cases of severe hypoglycaemia receiving emergency medical treatment in the Greater Copenhagen area [58].

Drug/substance	Age group, n (%)		
	All n = 141	Age < 50 years n = 77	Age ≥ 50 years n = 64
Alcohol.....	24 (17)	11 (14)	13 (20)
<i>Pharmaceutical drugs</i>			
Antidepressants.....	7 (5)	3 (4)	4 (6)
Benzodiazepines.....	5 (4)	2 (3)	3 (5)
Opiates.....	3 (2)	1 (1)	2 (3)
Neuroleptics.....	1 (1)	0 (0)	1 (2)
Barbiturates.....	1 (1)	1 (1)	0 (0)
All	17 (12)	7 (9)	10 (16)
<i>Illicit drugs</i>			
Marijuana.....	7 (5)	7 (9)	0 (0)
Amphetamine.....	1 (1)	1 (1)	0 (0)
All	8 (6)	8 (10)	0 (0)
Overall*	43 (31)	22 (29)	21 (33)

*) In 4% of samples both alcohol and drugs were detected.

hypoglycaemia rendering diabetic smokers more sensitive to glycogen depletion in the setting of repetitive hypoglycaemia. Secondly, nicotine affects brain metabolism and impairs cerebral glucose uptake (518). Thirdly, smoking could associate with severe hypoglycaemia by increasing the risk of peripheral neuropathy that per se is a marker of severe hypoglycaemia [13, 27]. The fact that both smoking and neuropathy were significant risk factors in a multivariate analysis [27] is, however, evidence against this explanation. Finally, the strong interaction between smoking and alcohol consumption [27] could indicate an overall effect of life-style, self-management, or compliance.

Use of alcohol

Consumption of excessive amounts of alcohol is a well-known risk factor for severe hypoglycaemia in insulin-treated diabetes [52]. Alcohol may promote the risk of severe hypoglycaemia by interfering with cognitive function and self-care [53], by compromising awareness of hypoglycaemic symptoms [54], and by reducing the hepatic production or mobilisation of carbohydrates during hypoglycaemia [55, 56]. Studies based on interviews have implicated alcohol in up to 19% of severe hypoglycaemic episodes [47-49, 57]. In our cross-sectional survey, alcohol consumption was not an independent risk factor for reporting severe hypoglycaemia, but there was a significantly elevated rate in subjects using both alcohol and tobacco [27]. We assessed the frequency of use of alcohol prior to episodes of insulin-induced severe hypoglycaemia receiving emergency medical care in the Greater Copenhagen area and identified alcohol in 17% of blood screens with a median concentration of ethanol of 11

mmol/l [58] (Table 4). The vast majority (111 of 141 (79%)) of the patients had type 1 diabetes. The result is in accordance with previous studies [47-49, 57] and suggests that alcohol may be implicated in a significant proportion of the most severe hypoglycaemic episodes.

Use or abuse of psychoactive drugs and substances

The use of psychoactive drugs – legal and illicit – has grown among younger generations and is now widely accepted practice [59-61], also in young people with type 1 diabetes [62]. Like alcohol, such substances can temporarily interrupt self-care [63] and increase the risk of accidents and trauma [64, 65]. As a major proportion of subjects with diabetes may be exposed to such drugs, the question arises whether psychoactive drugs might increase the risk of severe hypoglycaemia. In our case series of severe hypoglycaemia receiving emergency medical care we assessed and compared the frequencies of use of psychoactive drugs and alcohol prior to episodes of severe hypoglycaemia [58] using a thorough screening of blood samples [66] obtained during the episodes. Drugs were detected as frequently as alcohol, and among drugs illicit agents (mostly cannabis) accounted for a large proportion (Table 4). Overall, a psychoactive substance was identified in the blood screen in 33% of the cases. When limiting the analysis to the age group below 50 years, illicit agents were as commonly identified as alcohol (Table 4). The study was a case series without a control group and therefore no causal conclusions can be drawn. However, the result strongly suggests that drugs may be as significant as alcohol in promoting risk of severe hypoglycaemia and, furthermore, that diabetic people may be attracted by other psychoactive agents with less known association with severe hypoglycaemia than alcohol. There are no similar studies published but the frequency of drug identifications is similar to those obtained in trauma cohorts in which psychoactive drugs are well-established risk promoters [64-67].

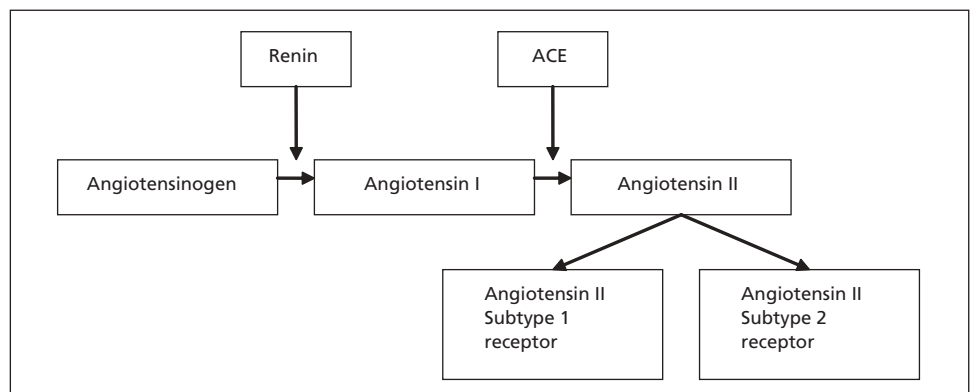
SEARCHING FOR NEW RISK FACTORS: THE RENIN-ANGIOTENSIN SYSTEM

Even though a large number of risk factors for severe hypoglycaemia in type 1 diabetes have been identified, they only explain a minor proportion of the variability in risk (estimated 10-15%). This underscores the relevance of searching for new risk markers.

The renin-angiotensin system (Figure 3) has traditionally been regarded as an endocrine system with its major impact on fluid and electrolyte homeostasis. During recent years, however, an increasing amount of evidence has demonstrated that intact renin-angiotensin systems are located in all highly oxidative organs including the brain and that, in addition to the well-known endocrine effects, the systems exert important para- and even autocrine effects [68]. Such effects include modulation of metabolism and resistance to noxious stimuli [69].

The primary substrate of the renin-angiotensin system is angiotensinogen (Figure 3), which is cleaved by renin to form biologically

Figure 3. The renin-angiotensin system.



inactive angiotensin I that in turn is cleaved by angiotensin-converting enzyme (ACE) to yield active angiotensin II. Angiotensin II exerts its effect via angiotensin II receptors, of which several subtypes have been identified. The subtype 1 receptor mediates fluid retention, hypertension and cell proliferation and is the target of angiotensin II receptor blockers. The more recently identified subtype 2 receptor mediates effects antagonistic to those of the subtype 1 receptor and the net effect of stimulation of the renin-angiotensin system is believed to depend on the balance between the activities of these two receptor subtypes [7]. ACE is a rather non-specific peptidase, which in addition to its main action on angiotensin I has activity on a number of other substrates such as bradykinin which is degraded to inactive peptides [71].

The activity of the endocrine renin-angiotensin system is under acute influence of posture, fluid and electrolyte status and under more chronic influence by a number of genetic and other factors. Thus, the plasma renin activity and angiotensin II concentration increase rapidly in response to change from supine to upright position, hypovolaemia and hypoglycaemia [72]. On the contrary, plasma angiotensinogen concentration is quite constant, and is influenced by a number of polymorphisms in the *angiotensinogen* gene [73]. Likewise, serum ACE activity is stable and under major control of the *I (insertion)/D (deletion)* polymorphism of the *ACE* gene, of which the *I*-allele confers low tissue and blood activity of ACE [74, 75], probably due to its production of an ACE protein that has only one of its two putative active sites [76]. The *ACE I/D* polymorphism has been reported to explain up to 40% of the variation in serum ACE activity [74]. Furthermore, the *D*-allele is associated with increased in-vivo degradation rate of bradykinin [71]. The *I/D* polymorphism has been implicated in the promotion of a variety of pathological conditions including late diabetic complications [77] and myocardial hypertrophy in heart failure [78]. Common genetic variations have also been identified in the *angiotensin II receptor subtype 1* and *2* genes. In the *subtype 1 receptor* a common single nucleotide polymorphism has been localised to position 1164 [79]. The functional significance of this variation remains, however, uncertain [79]. In the *subtype 2 receptor*, which is located on the X-chromosome, a single nucleotide polymorphism is found at position 1675. The *A*-allele is associated with reduced transcription of the gene [8] and has been associated with development of cardiac hypertrophy in hypertensive subjects [81]. Genetic variation in the *angiotensin II receptors* may interfere with the balance between the receptors and this may become more significant in conditions with activated renin-angiotensin system such as diabetes [82].

THE HYPOTHESIS

The *I/D* polymorphism of the *ACE* gene has recently been associated with performance capacity of endurance athletes [83]. Thus, an increased frequency of the *I* allele has been demonstrated in high-altitude mountaineers [83], in Australian Olympic rowers [84], and in British Olympic long-distance runners [85]. These groups of athletes are resulting from a year-long selection for optimal endurance performance and the studies indicate that the *I* allele, and thereby low ACE activity, may be favourable for endurance performance capacity. An important factor for success in competitive endurance sports is the ability to maintain functional capacity despite of severely impaired fuel availability and recent research has implicated fuel deprivation of the brain as an important limiting factor for endurance performance [86].

Patients with type 1 diabetes regularly experience hypoglycaemic episodes during daily life. The majority of these episodes remain unrecognised by the patient [87, 88]. The ability to maintain optimal performance during these silent hypoglycaemic episodes, in particular in terms of cognitive function, is crucial in order to avoid interruption of daily activities and development of severe hypoglycaemia before a normal glucose level is restored by a pre-planned snack or meal or by dissipation of the insulin effect. Like a bout of endurance

exercise a hypoglycaemic episode is an event of relatively impaired fuel availability. Just as in competitive endurance athletes the capacity of type 1 diabetic subjects to maintain function despite of limited substrate availability may be related to ACE activity. This led us to hypothesise that high ACE activity may be a marker of the risk of severe hypoglycaemia in these patients.

ACE AND RATE OF SEVERE HYPOGLYCAEMIA

Based on recall of severe hypoglycaemia, we tested the hypothesis in a cohort of consecutive patients with type 1 diabetes untreated with ACE inhibitors or angiotensin II receptor antagonists [10]. In accordance with our hypothesis we found that subjects with the *DD* genotype had a relative rate of 3.2 of severe hypoglycaemia in the preceding two-year period compared to patients with the *II* genotype. A significant relationship between serum ACE activity and the rate of severe hypoglycaemia was observed with a relative rate of 1.4 per ten U/ml increment of serum ACE, corresponding to a 3.5-fold increased rate in subjects in the upper quartile compared to subjects with serum ACE in the lowest quartile (Figure 4A). A multiple regression analysis showed that the effect of the *ACE* genotype was explained by its influence on serum ACE and that the only other markers of severe hypoglycaemia were impaired hypoglycaemia awareness, undetectable C-peptide, and duration of diabetes. The influence of serum ACE on the risk of severe hypoglycaemia was most pronounced in patients with other risk factors such as im-

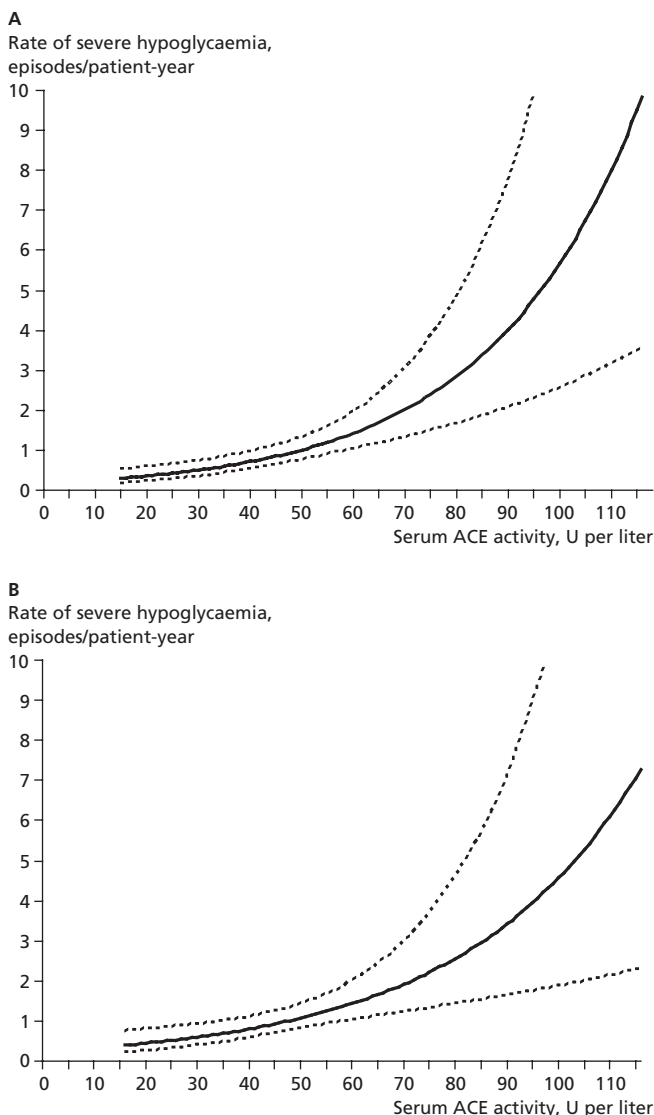


Figure 4. Relationship between serum ACE activity and rate of severe hypoglycaemia assessed retrospectively (panel A; n=213) (10) and prospectively (panel B; n=171) (16). Estimate with 95% confidence limits.

paired awareness (Figure 5A), undetectable C-peptide (Figure 5B) and long duration of diabetes (Figure 5C). There was no significant relationship between ACE genotype or serum ACE and residual beta cell function or self-estimated state of hypoglycaemia awareness.

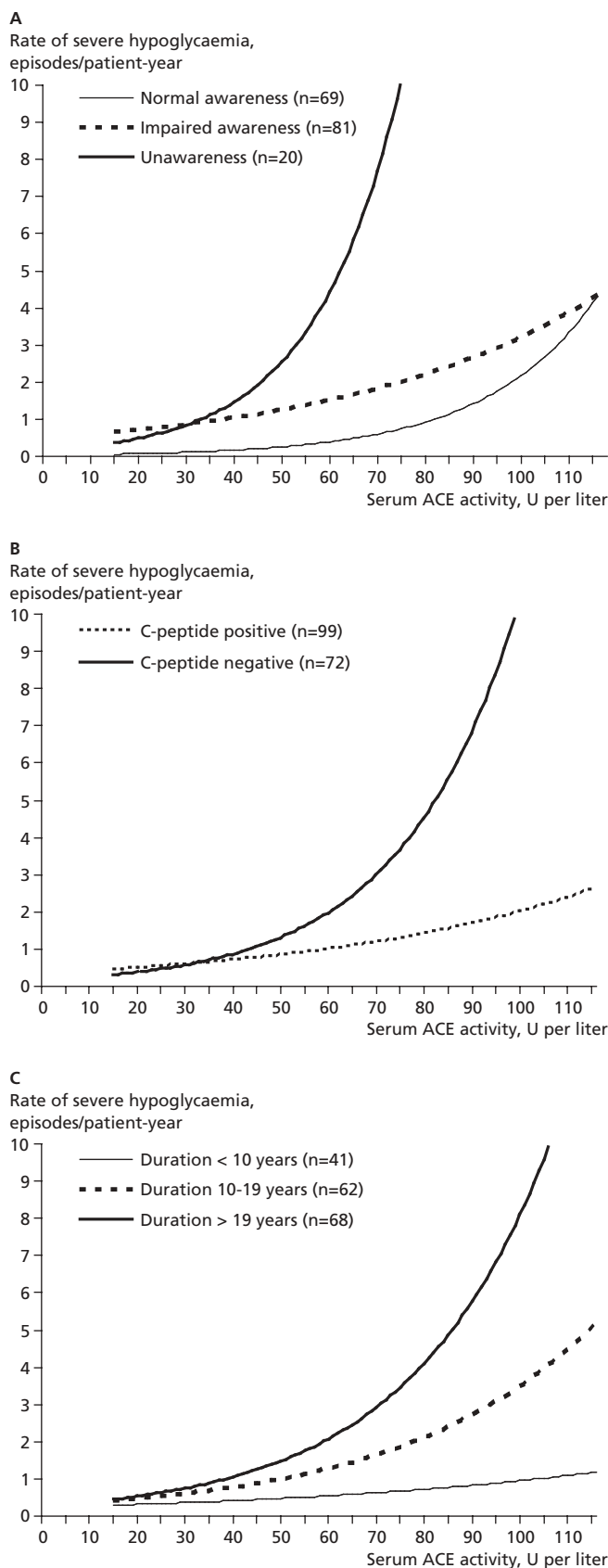


Figure 5. Relationship between serum ACE activity and prospectively reported rate of severe hypoglycaemia (n=171) [16] according to self-estimated hypoglycaemia awareness (panel A), C-peptide status (panel B), and duration of diabetes (panel C).

Subsequently, we reassessed the association between ACE activity and severe hypoglycaemia in a one-year prospective study of 171 subjects from the original cohort untreated with ACE inhibitors and angiotensin II receptor blockers [16]. This study confirmed the result of the retrospective study [10] by demonstrating a positive relationship between serum ACE activity and rate of severe hypoglycaemia with a relative rate of 2.7 of subjects in the upper quartile of ACE activity compared to the lowest quartile (Figure 4B). A similar relationship was observed for the subset of episodes with coma or need for parenteral treatment (relative rate in upper ACE quartile compared to lowest quartile: 2.9). The impact of serum ACE activity was most pronounced in C-peptide negative subjects (relative rate in upper ACE quartile compared to lowest quartile: 4.2), and in this subgroup carriers of the D allele of the ACE gene had higher rates of severe hypoglycaemia compared to the group homozygous for the I allele. In a multiple regression analysis high serum ACE activity and impaired awareness of hypoglycaemia were identified as the only significant predictors of severe hypoglycaemia. An association between serum ACE activity and rate of severe hypoglycaemia with a relative rate of 3.5 in the upper serum ACE quartile has recently been reported in a large Scottish cohort with type 1 diabetes [89] resembling ours [16]. Furthermore, a study on a Swedish cohort of children reported an even stronger association with 6-fold increased rate in the group with serum ACE activity above median [90]. In contrast, an Australian post-hoc study on a cohort of children, in which severe hypoglycaemia was not a predefined endpoint and events were not validated, failed to demonstrate any association with the I/D polymorphism of the ACE gene [91]. ACE activity was not measured in that study.

RENIN-ANGIOTENSIN SYSTEM ACTIVITY AND RATE OF SEVERE HYPOGLYCAEMIA

The association of high ACE activity with severe hypoglycaemia may hypothetically be mediated through generally increased renin-angiotensin system activity acting through either angiotensin II receptor subtype, or through ACE-mediated reduction of kinin levels. In our prospective study we explored which of these pathways were involved in promoting risk of severe hypoglycaemia by examining plasma angiotensinogen concentration, functional polymorphic variations in the *angiotensinogen* gene as well as polymorphisms in *angiotensin II receptor subtypes 1 and 2* and *bradykinin 2 receptor* genes [92]. This study identified two novel risk factors for severe hypoglycaemia in the renin-angiotensin system: plasma angiotensinogen concentration in the upper quartile (relative rate 3.1 vs. lower quartile) and homozygosity or hemizygoty for the A-allele of the *AT₂R 1675G>A* polymorphism (relative rate 2.5 vs. non-carriers) in addition to high serum ACE activity (Table 5). There was no effect of *angiotensinogen*, *AT₁R*, or *bradykinin 2 receptor* genotypes (Table 5). A post-hoc analysis of the data showed that presence of all three risk factors implied a positive predictive value of 100% (CI: 39%-100%) for reporting ≥ 5 episodes and absence of all three factors implied a negative predictive value of 91% (CI: 81%-97%) for reporting > 1 episode of severe hypoglycaemia per year (Figure 6). These results obviously need to be confirmed prospectively in an independent cohort. On multiple regression analysis increasing number of renin-angiotensin system-related risk factors together with impaired hypoglycaemia awareness were independently associated with severe hypoglycaemia.

RENIN-ANGIOTENSIN SYSTEM INHIBITION AND RATE OF SEVERE HYPOGLYCAEMIA

Further observations suggest that pharmacological inhibition of the renin-angiotensin system might reduce risk of severe hypoglycaemia. Thus, in our multicentre survey [27] the group of patients treated with antihypertensive agents without direct inhibitory effect on the renin-angiotensin system reported an elevated rate of severe hypoglycaemia (relative rate 2.5 vs. untreated patients) in marked

Table 5. Univariate analyses of associations of renin-angiotensin system-related factors with prospectively recorded rate of severe hypoglycaemia in 171 patients with type 1 diabetes untreated with ACE inhibitors and angiotensin receptor blockers [92].

Variable	Severe hypoglycemia Relative rate* (95% CI)	p	Overall p
Angiotensinogen M235T			0.69
MT	1.1 (0.6-2.2)	0.71	
TT	1.5 (0.6-3.9)	0.40	
Angiotensinogen T174M			0.13
TM	1.7 (0.8-2.7)	0.16	
MM	0.2 (0.02-2.3)	0.20	
Angiotensinogen A(-6)G			0.42
GA	1.4 (0.8-2.7)	0.28	
AA	1.7 (0.7-4.2)	0.27	
Angiotensinogen C(-20)A			0.25
AC	1.7 (0.9-3.3)	0.11	
CC	0.9 (0.2-4.2)	0.89	
Plasma angiotensinogen			0.0045†
2nd quartile	1.1 (0.5-2.6)	0.76	
3rd quartile	1.0 (0.4-2.2)	0.89	
4th quartile	3.1 (1.4-6.8)	0.0042‡	
ACE I/D			0.40
ID	1.5 (0.7-3.3)	0.27	
DD	1.5 (0.7-3.3)	0.27	
Serum ACE activity			0.0048†
2nd quartile	0.73 (0.31-1.7)	0.47	
3rd quartile	1.2 (0.52-2.6)	0.73	
4th quartile	2.9 (1.3-6.2)	0.0083‡	
AT1R 1166A/C			0.68
AC	1.1 (0.4-3.2)	0.81	
CC	1.4 (0.5-4.0)	0.49	
AT2R 1675G/A			0.028
GA	1.3 (0.6-2.9)	0.50	
A(A)	2.5 (1.4-5.0)	0.0093§	

CI = confidence interval.

*) Relative rate values represent: all genotypes: compared to wild type (ACE genotype: II); angiotensinogen- and ACE quartiles: compared to 1st quartile.

We also show which of the 9 different covariates in the renin-angiotensin system were significantly associated with risk of severe hypoglycemia even after correction for multiple comparison using the Bonferroni method: † For the 9 primary comparisons p-values should be <0.0056 (<0.05/9), ‡ for 3 post hoc comparisons p-values should be <0.017 (0.05/3), while § for 2 post hoc comparisons p-values should be <0.025 (0.05/2).

contrast to the relative rate of 0.8-1.0 of the otherwise comparable groups treated with antihypertensive regimens including ACE inhibitors or angiotensin II receptor antagonists, which was lower than expected from diabetes duration [27]. The lower rate in subjects treated with agents with inhibitory effect on the renin-angiotensin system compared to subjects on other antihypertensive regimens suggests a beneficial effect of ACE inhibitors or angiotensin II receptor antagonists on risk of severe hypoglycaemia in type 1 diabetes. The finding contradicts the disputed association between ACE inhibitor treatment and increased risk of severe hypoglycaemia, which has been reported in some early registry-based studies [93, 94]. The results of these studies are probably explained by differences in duration of diabetes which is typically much longer in ACE inhibitor-treated patients than in untreated patients, and which may introduce confounders such as differences in state of awareness and C-peptide status between treated and untreated groups. In accordance, no increased risk of severe hypoglycaemia has been reported in a controlled trial [95].

RENIN-ANGIOTENSIN SYSTEM AND VULNERABILITY TO METABOLIC STRESS IN ANIMALS

The concept that high renin-angiotensin system activity might be associated with reduced capability to maintain cognitive function during hypoglycaemia is supported by substantial data from animal experiments. Thus, blocking the renin-angiotensin system by an ACE inhibitor or angiotensin II receptor blocker treatment increases neuronal resistance to ischaemia [96, 97]. Conversely, in-vivo studies in rodents subjected to experimental stroke have shown that animals overexpressing the *angiotensinogen* gene [97] develop in-

creased infarction volume and that intracerebroventricular infusion of angiotensin II results in increased brain damage following exposure to hypoxia [96]. Knockout of the *angiotensin II subtype 1 receptor* gene or blockade of the receptor, which like other components of the renin-angiotensin system are abundant throughout the brain, results in reduced infarction size [97] indicating that the effect of increased renin-angiotensin system activity is probably mediated by this receptor. Conversely, stimulation of the subtype 2 receptor protects against ischaemic infarction [98] and this receptor subtype – in contrast to the subtype 1 receptor – is upregulated after exposure of the rat brain to transient ischaemia [99]. This suggests a beneficial effect of the subtype 2 receptor in protection against ischaemic stress and is in accordance with our finding of increased susceptibility to severe hypoglycaemia with the A-allele of the *subtype 2 receptor 1675 G>A* polymorphism, which is associated with reduced receptor function [92].

THE RENIN-ANGIOTENSIN SYSTEM AND RESPONSES TO HYPOGLYCAEMIA IN MAN

In accordance with the pronounced individual differences in rate of severe hypoglycaemia wide individual differences in the ability to maintain cognitive function during experimental hypoglycaemia have been reported [100]. We tested in healthy humans the influence of ACE activity on the ability to maintain cognitive function during hypoglycaemia [101]. Two groups each consisting of eight healthy subjects selected by either particularly high or low serum ACE activity were subjected to hypoglycaemia and euglycaemia in a cross-over study. Hypoglycaemia was induced by repetitive subcutaneous insulin injections without concomitant glucose infusion. The rationale for not applying conventional hyperinsulinaemic hypoglycaemic clamping methods was that differences in responses could potentially occur in depth of the hypoglycaemic response and such a difference would be abolished by glucose infusion during the experiment. Cognitive function and auditory evoked potentials, as well as hypoglycaemic symptoms, hormonal counterregulatory re-

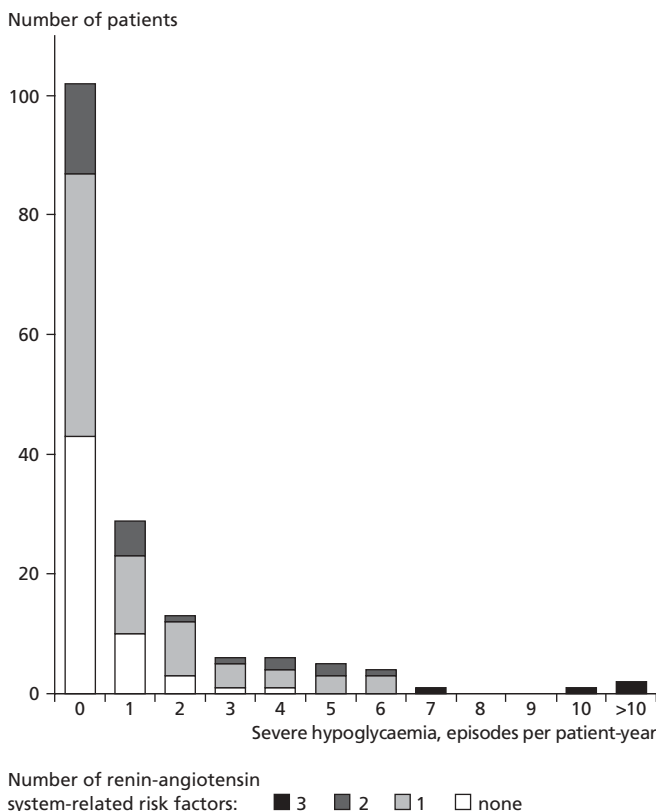


Figure 6. Distribution of renin-angiotensin system-related risk factors according to prospectively reported rate of severe hypoglycaemia (n=171) [92].

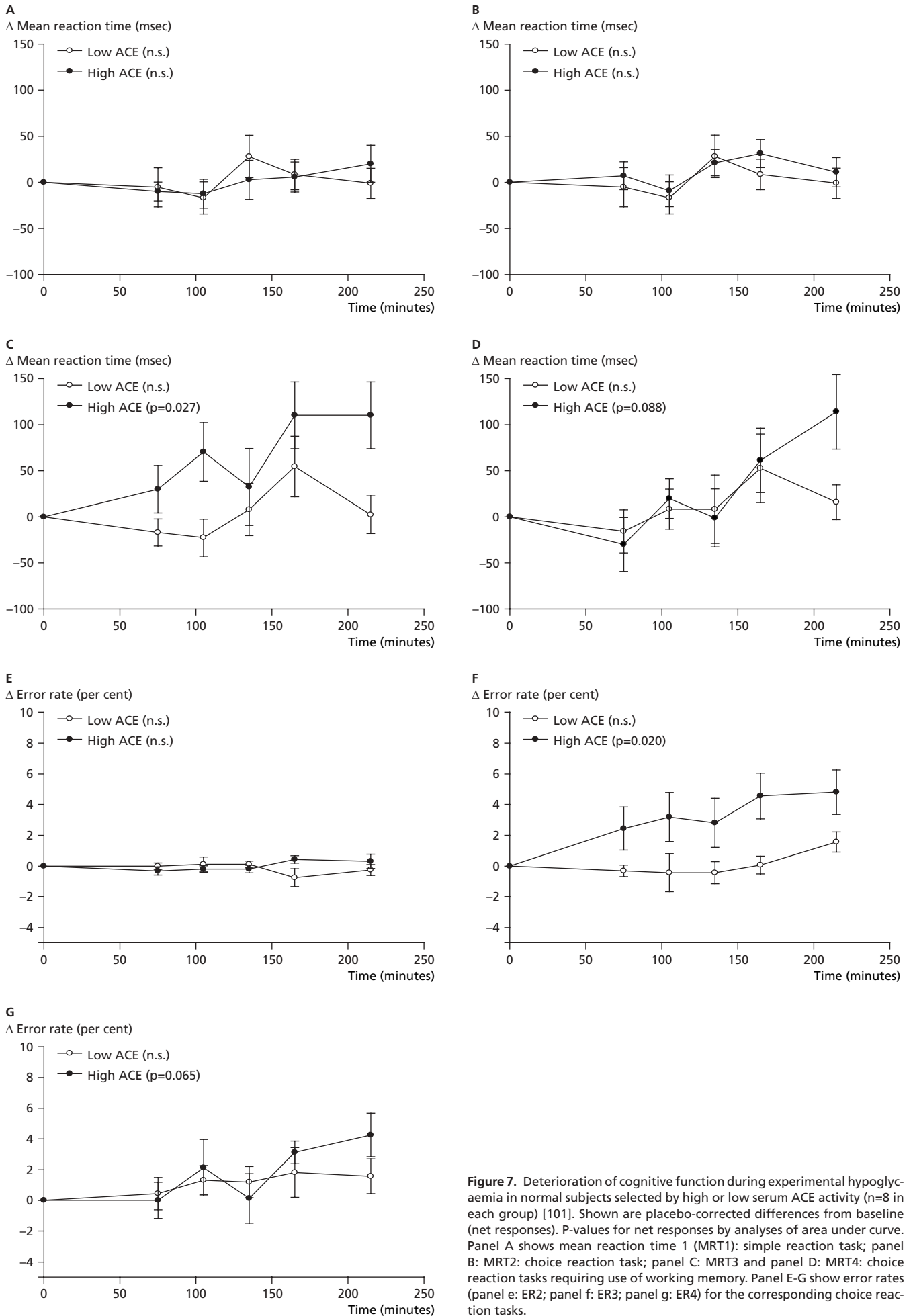


Figure 7. Deterioration of cognitive function during experimental hypoglycaemia in normal subjects selected by high or low serum ACE activity (n=8 in each group) [101]. Shown are placebo-corrected differences from baseline (net responses). P-values for net responses by analyses of area under curve. Panel A shows mean reaction time 1 (MRT1): simple reaction task; panel B: MRT2: choice reaction task; panel C: MRT3 and panel D: MRT4: choice reaction tasks requiring use of working memory. Panel E-G show error rates (panel e: ER2; panel f: ER3; panel g: ER4) for the corresponding choice reaction tasks.

sponses, renin-angiotensin system responses, and concentrations of circulating substrates were assessed during the experiments. Despite a similar hypoglycaemic stimulus in the two groups reaching a blood glucose nadir of 2.7 mmol/l, only the group with high ACE activity showed significant deterioration in cognitive performance (Figure 7) during hypoglycaemia [101]. The cognitive dysfunction in the high ACE group, which was only observed in the most complex reaction time tasks requiring the use of working memory and which affected both speed and precision, persisted even after normalization of plasma glucose concentrations (Figure 7). The group with high ACE activity also continued to report elevated hypoglycaemic symptom scores following recovery of glucose levels (Figure 8). There were no major differences in responses of counterregulatory hormones between the groups. More brisk alterations of concentrations of substrates were observed in the group with low ACE activity. Preliminary data from our laboratory suggest that the renin-angiotensin system also affects hypoglycaemic cognitive function in type 1 diabetes. Thus, a group of subjects selected by high renin-angiotensin system activity deteriorated significantly in performance of complex reaction time tasks in contrast to a group with particularly low spontaneous renin-angiotensin system activity [102].

In accordance with this demonstration of an effect that is independent of known protective mechanisms against hypoglycaemia we found only a weak statistical association between ACE activity or renin-angiotensin system activity and awareness in our cohort studies [16, 92]. ACE activity or renin-angiotensin system activity remained significant predictors of severe hypoglycaemia in multiple regression analyses including awareness level emphasizing the existence of independent effects. Likewise, there was no association between renin-angiotensin system activity and potential markers for counterregulatory capacity such as C-peptide concentration, HbA1c and duration of diabetes [15, 92].

Previous experimental human studies have indirectly suggested an association of high renin-angiotensin system activity with vulnerability to hypoglycaemia. Hirsch et al reported that subjects with type 1 diabetes had more intense symptoms and needed to mount greater counterregulatory responses during a hypoglycaemic clamp in the standing position compared with the supine position [103]. The authors ascribe their finding to increased hypoglycaemia awareness in the standing position but it could as well be explained by activation of the renin-angiotensin system rendering the subjects more sensitive to the effect of the hypoglycaemic challenge. The best way to discriminate between these interpretations would be to assess cognitive function, which was not done in that study. In accordance with the alternative interpretation, pharmacological blockade of ACE activity by ACE inhibitor treatment has been reported to attenuate counterregulatory responses in terms of catecholamines [104] and ACTH [105] and to reduce the glucose infusion rate necessary to maintain the glucose level in hypoglycaemic clamp studies [106] in healthy subjects. Also angiotensin II receptor blockade with losartan reduces symptoms and counterregulatory responses during hypoglycaemia in healthy subjects [107, 108]. Together these studies suggest a beneficial effect of renin-angiotensin system blockade on maintenance of metabolism during hypoglycaemia.

POSSIBLE MECHANISMS OF ACTION

An effect of the renin-angiotensin system on brain function during hypoglycaemia could be caused by an influence on either supply or demand of substrate, or both. The circulating renin-angiotensin system may influence supply of substrates to the brain by modulating blood flow that is crucial for maintenance of substrate supply during hypoglycaemia. During experimental hypoglycaemia with cognitive impairment cerebral blood flow increases and such a flow compensation is thought to conserve glucose supply to the brain [109]. Increasing cerebral blood flow pharmacologically during hypoglycaemia in healthy humans results in blunted epinephrine

and hypoglycaemic symptom responses [110] probably by enhancing substrate supply. The cerebral vasodilatory response to hypoglycaemia might be blunted in subjects with high basal activity of the renin-angiotensin system.

Alternatively, the paracrine renin-angiotensin system, which is abundant in the brain, may influence the cerebral metabolism of substrates [69]. In our study, the normal subjects with low ACE activity showed more significant alterations in circulating substrates during hypoglycaemia than those with high activity [101]. This suggests a superior capacity of subjects with low ACE activity to switch to alternative substrates such as lactate, which conserves brain func-

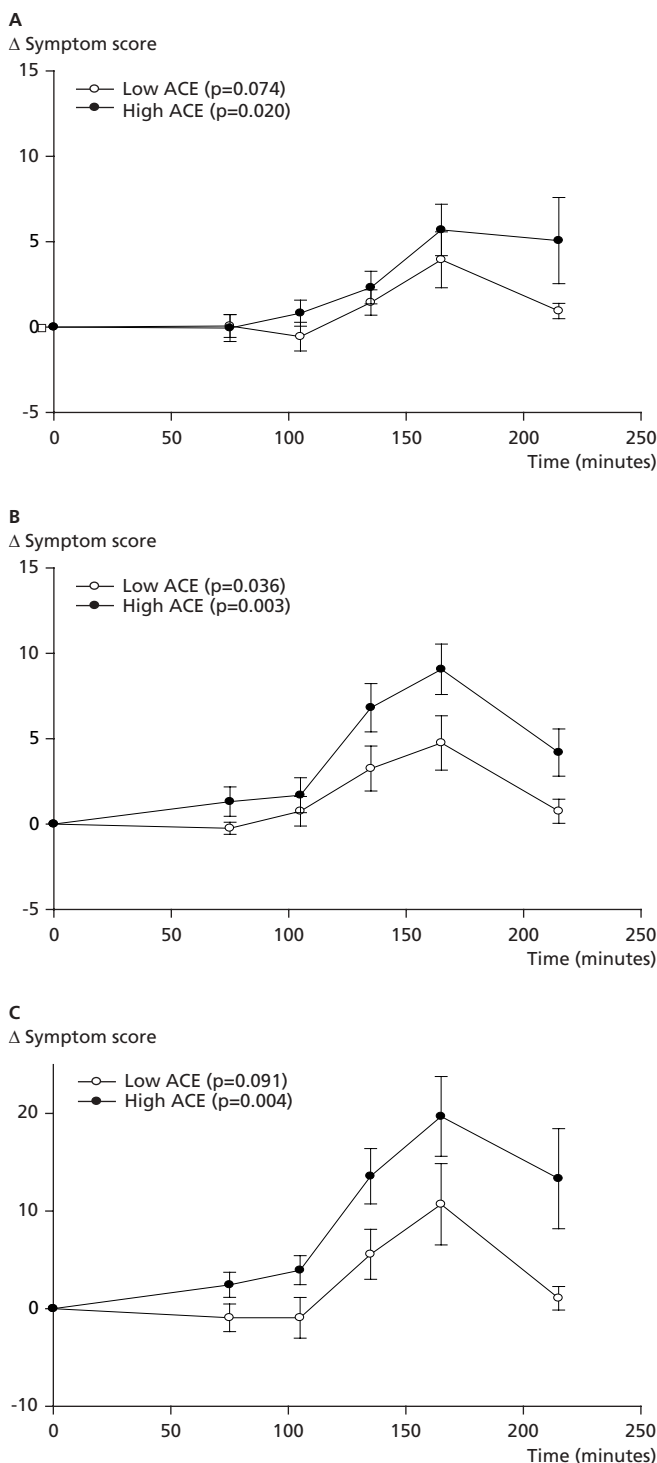


Figure 8. Symptomatic responses during experimental hypoglycaemia in normal subjects selected by high or low serum ACE activity (n=8 in each group) [101]. Shown are mean (SEM) net-responses (placebo-corrected Δ -values). Panel A: autonomic symptom score; panel B: neuroglycopenic symptom score; panel C: total symptom score.

tion during hypoglycaemia [111]. In accordance, in experimental heart failure ACE inhibition improves myocardial energy efficiency [112] and knockout of the *angiotensin II subtype 1 receptor* gene is associated with conservation of ATP in neurons exposed to ischaemia [97]. In addition, experimental data implicate the renin-angiotensin system in modulation of peripheral glucose metabolism. Thus, inhibition of the angiotensin II receptor subtype 1 increases insulin-induced glucose uptake in rat muscle possibly by increasing glucose transporter protein GLUT-4 [113]. A low renin-angiotensin system activity could thereby be beneficial for glycaemic stability in type 1 diabetes and for replenishment of energy stores after hypoglycaemia, improving the metabolic resistance to subsequent episodes.

CONCLUSION AND FUTURE PERSPECTIVES

Our studies confirm that severe hypoglycaemia is still a major clinical problem in type 1 diabetes with a rate comparable to that of the pre-DCCT era despite significant improvement of insulin therapy and diabetes care – and will probably remain so as long as a closed loop system for insulin treatment is not a standard therapeutic option. The individual susceptibility to severe hypoglycaemia is highly varying and conventional risk factors – with major contribution from hypoglycaemia awareness – only account for a limited part of this variation. The use of psychoactive substances may be as significant as alcohol for promotion of risk of severe hypoglycaemia and until this is confirmed by case-control studies the diabetes team should explore the use of such substances in patients prone to severe hypoglycaemia.

Elevated renin-angiotensin system activity is a novel predictor of risk of severe hypoglycaemia in type 1 diabetes with potential clinical significance. The clinical implications of this finding which, however, must await additional independent confirmation, include prediction and possibly some prevention of severe hypoglycaemia. By combining three sequential renin-angiotensin system-related risk factors in a common model both subjects at low and at high risk within a one-year period were identified. Although plasma angiotensinogen and serum ACE activity are under significant genetic control and stable over time the use of these parameters for prediction of severe hypoglycaemia over longer periods will need further prospective testing including serial blood sampling. An evaluation of renin-angiotensin system activity may – together with assessment of other risk factors – contribute to rational individualised setting of glycaemic targets and thereby open for prevention of severe hypoglycaemia.

The renin-angiotensin system-based stratification system identified a small number of type 1 diabetic patients with a novel extreme phenotype characterised by 1) generally elevated renin-angiotensin system activity and 2) an incapacitating high rate of severe hypoglycaemia. Such subjects may benefit from pharmacological blockade of the renin-angiotensin system by ACE inhibitors or angiotensin II receptor blockers or even renin blockers. This should be addressed in controlled trials. Ultimately, newly diagnosed patients with type 1 diabetes can be screened routinely for renin-angiotensin system activity and genetics and on the basis of that, some patients might be placed on renin-angiotensin system blocking therapy in order to prevent severe hypoglycaemia. In addition, this might potentially reduce their risk of developing late complications.

Our studies may also contribute to the explanation of the association between ACE genotypes and endurance performance capacity suggesting a beneficial effect of low ACE activity. As our study shows that cognitive function declines significantly in healthy subjects with high ACE activity exposed to a moderate degree of substrate deficiency for a relatively short duration, ACE activity (or renin-angiotensin system activity) may be an important determinant of development of fatigue during endurance sports with exposure to severe and long-lasting fuel deprivation. Thus, the poorly understood phe-

nomenon central fatigue is regarded as an important limiting factor of endurance performance and has been attributed to impairment of brain function due to exhaustion or fuel starvation. This phenomenon may well be influenced by ACE or renin-angiotensin system activity. Future research should be directed towards exploration of the cellular mechanisms influenced by the renin-angiotensin system in fuel exhaustion.

We have introduced genetics as a useful tool in hypoglycaemia research and the results obtained encourage the exploration of other candidate genes for susceptibility to severe hypoglycaemia. Such genes might be identified in the known counterregulatory pathways (e.g. beta receptors) or in association with awareness or in other metabolic pathways, which may be important for performance during glucose deficiency. Well-powered genetic linkage studies are needed for exploration of these issues.

SUMMARY

Hypoglycaemia is an unavoidable side effect to insulin therapy of diabetes. In daily life some hypoglycaemic episodes are recognised by the patients and corrected by ingestion of glucose, but occasionally unrecognised episodes progress into severe hypoglycaemia with cognitive impairment and the need for assistance from other persons in order to manage the situation. Such episodes represent the most feared side effect to insulin treatment and are regarded as the major limiting factor for achievement of recommended glycaemic targets in type 1 diabetes. The series of studies that constitute this thesis was conducted to assess the significance of severe hypoglycaemia as a clinical problem in the type 1 diabetic population, to evaluate the impact of known risk factors on occurrence of severe hypoglycaemia, and to identify new markers that could contribute to improved prediction of, and inspire to novel preventive measures of, severe hypoglycaemia.

Our studies confirm that severe hypoglycaemia is still a major clinical problem in type 1 diabetes. The individual susceptibility to severe hypoglycaemia is highly varying and conventional risk factors – with major contribution from hypoglycaemia unawareness – only account for a limited part of this variation. Results from a case-series suggest that the use of psychoactive substances may be as significant as alcohol for promotion of risk of severe hypoglycaemia – a finding which needs to be confirmed by case-control studies.

We identified elevated renin-angiotensin system activity as a novel predictor of risk of severe hypoglycaemia in type 1 diabetes with potential clinical significance. Thus, three sequential renin-angiotensin system-related risk factors were associated with severe hypoglycaemia, and by including these factors in a common model both subjects at low and at high risk within a one-year period were identified. Preliminary data suggest that this is explained by impaired capability of subjects with high renin-angiotensin system activity to maintain cognitive function during hypoglycaemia. The clinical implications of this finding which, however, must await additional independent confirmation, include prediction and possibly some prevention of severe hypoglycaemia. An evaluation of renin-angiotensin system activity may – together with assessment of other risk factors – contribute to rational individualized setting of glycaemic targets and thereby open for prevention of severe hypoglycaemia. Furthermore, subjects with elevated renin-angiotensin system activity and a high rate of severe hypoglycaemia might benefit from pharmacological blockade of the renin-angiotensin system by ACE inhibitors or angiotensin II receptor blockers or even renin blockers. This should be addressed in controlled trials.

LIST OF ABBREVIATIONS:

ACE: angiotensin-converting enzyme
ARB: angiotensin II receptor blocker
ATI: angiotensin I
ATII: angiotensin II
DCCT: The Diabetes Control and Complications Trial
RR: relative rate

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