Delay in Onset of Awareness of Acute Hypoglycemia and of Restoration of Cognitive Performance During Recovery

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OBJECTIVE — To examine the time course for the onset of, and recovery from, acute hypoglycemia in healthy subjects.

RESEARCH DESIGN AND METHODS — Eight healthy male volunteers were studied on 2 occasions in random order using a hyperinsulinemic (1.5 mU · kg^{-1} · min^{-1}) glucose clamp technique. During control studies, euglycemia (5.01 ± 0.02 mmol/l) was maintained for 225 ± 3 min. On the other occasion, after a euglycemic baseline period, arterialized plasma glucose was allowed to fall rapidly to 2.65 ± 0.02 mmol/l, then maintained at this nadir for 90 min before euglycemia was rapidly restored.

RESULTS — Cognitive function assessed by a battery of sensitive tests (4-choice reaction time, Stroop word, and color-word test) became impaired immediately at onset of hypoglycemia ($P < 0.05$ for all in the hypoglycemic study vs. those in the euglycemic study). Counterregulatory hormone responses (epinephrine, norepinephrine, glucagon, cortisol, and growth hormone) and symptomatic awareness of hypoglycemia (assessed by a questionnaire) were relatively delayed, being detected 20 min after the onset of hypoglycemia. There was no diminution (adaptation) of any responses, cognitive, humoral, or symptomatic, during sustained hypoglycemia. During recovery, the 4-choice reaction time continued to be abnormal even after resolution of symptomatic awareness ($P = 0.025$).

CONCLUSIONS — During hypoglycemia, cognitive performance may become impaired before symptomatic awareness. During recovery from hypoglycemia, recovery of cognitive function lags behind the restoration of glucose levels and resolution of symptoms. Our findings have implications for the design of studies examining experimental hypoglycemia and need to be investigated in people with diabetes.

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Hypoglycemia is the most important acute complication of the insulin treatment of diabetes and is feared by patients with diabetes as much as chronic complications, such as nephropathy and retinopathy (1). Hypoglycemia or even fear of hypoglycemia may limit the achievement of the good glycemic control necessary to prevent these chronic complications, thus imposing significant medical and social costs. Some patients with diabetes develop significantly impaired counterregulatory responses to hypoglycemia, with associated loss of awareness of their low blood glucose level. This loss of protective symptomatic responses significantly increases the risk of severe hypoglycemia (2,3).

For good defense against severe hypoglycemia, it is important that symptomatic responses precede cognitive impairment as plasma glucose falls. This hierarchy has been determined in several studies using a modification of the hyperinsulinemic glucose clamp in which plasma glucose is reduced sequentially, typically in steps of 40- to 90-min durations, during each of which responses to hypoglycemia are measured 1 or 2 times (4–7). There are few data on the speed or sequence of onset of the different responses to a given plasma glucose level.

Kerr et al. (8,9) demonstrated that symptoms of hypoglycemia may diminish during sustained mild hypoglycemia despite a continued rise in hormonal responses in healthy and type 1 diabetic subjects. Other studies have found no such diminution of responses (10). Finally, few studies have directly addressed the speed and order of recovery from hypoglycemia-induced cognitive dysfunction, although this may also be relevant in interpreting experimental studies of hypoglycemia.

These issues have implications for the design and interpretation of studies examining acute hypoglycemia. Slow-fall hypoglycemic challenges have been widely used in hypoglycemia research to examine the glucose levels at which responses to hypoglycemia develop, but do not allow the temporal course of symptomatic, neurohumoral, and cognitive responses to a more acute hypoglycemia to be determined. We set out to examine whether the timing of the responses to acute hypoglycemia correspond exactly to the onset and duration of the low blood glucose. We also wished to see whether these responses might adapt (i.e., diminish) during sustained hypoglycemia. We have used a single-step exposure to a rapid-onset 90-min hypoglycemic challenge in healthy volunteers to examine these questions.

RESEARCH DESIGN AND METHODS

— We recruited 8 healthy male volunteers, mean age 28.5 ± 3.6 years and mean BMI 26.8 ± 4.4 kg/m^2.

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Abbreviations: EU, euglycemic studies; HYPO, hypoglycemic studies.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

None of the men had any chronic diseases, intercurrent illnesses, personal or family history of diabetes, or were on any medications. The protocol was approved by the Kings Healthcare Ethics Committee. All subjects gave written informed consent and were offered $75 in payment for participation.

Subjects were studied on 2 occasions in random order (1 hypoglycemic study [HYPO] and 1 euglycemic study [EU]), being blinded to the order of the studies and their plasma glucose level at all times during these studies. Studies were identical other than the glucose profiles, as detailed below. Subjects were admitted to the investigation ward at 8:00 A.M. after an overnight fast, having refrained from alcohol and tobacco for 24 h. Using intradermal lidocaine for local anesthesia, 2 intravenous cannulae were placed in the nondominant arm. One of these was positioned in a retrograde fashion in a distal hand or wrist vein. This hand was then rested in a hot box containing air warmed to 55°C to arterialize venous blood (11). This cannula was used for sampling arterialized blood for measurement of plasma glucose, intermediary metabolites, and counterregulatory hormones. The second cannula was placed more proximally in the antecubital fossa for infusion of insulin and glucose.

Not less than 40 min after the insertion of cannulae, baseline blood tests were sampled and a primed continuous infusion of 1.5 mU · kg^{-1} · min^{-1} of soluble insulin (Actrapid; Novo Nordisk, Crawley, U.K.) in a 4% autologous blood solution was started. A variable infusion of 20% glucose (Clintec Nutrition, Slough, U.K.) was given simultaneously and adjusted to maintain target glucose levels. Samples of arterialized blood were drawn every 5 min for bedside estimation of arterial plasma glucose levels using a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

During control studies, glucose levels were maintained at 5 mmol/l for 225 ± 3 min (EU). During the other study (HYPO), glucose levels were controlled at 5 mmol/l for the first 50 min of the study. The dextrose infusion was then temporarily discontinued so that glucose levels fell rapidly to 2.6 mmol/l (taking 18.1 ± 1.6 min) where they were maintained for an additional 90 min. Euglycemia was then rapidly restored. Hormone responses, symptom questionnaires, and cognitive test batteries were administered at regular intervals throughout both studies. In HYPO studies, complete data sets were collected as follows: at 0, 20, and 40 min (when the subjects were euglycemic); immediately upon achieving the hypoglycemic nadir; at 20, 40, 60, and 90 min after achieving the nadir (during continued hypoglycemia); and both immediately and 20 min after restoration of euglycemia. Data collections were made at equivalent time points in the EU studies.

Symptoms were assessed by the use of a questionnaire as previously described, asking subjects to rank sweating, warmth, palpitations, tingling, anxiety, trembling, hunger, blurred vision, tiredness/drowsiness, confusion, weakness, headache, difficulty in speaking, dizziness, and irritability individually on a linear analog scale. Autonomic symptom scores were derived from the first 7 symptoms listed, and neuroglycopenic scores from the latter 8 symptoms, with individual symptoms being ranked by subjects from 1 (absent) to 7 (maximal) (12). For ease of interpretation, these scores were subsequently adjusted to a scale of 0–6. Subjects were also asked simply whether they felt abnormal or not and to rank this from 1 (mildly abnormal) to 10 (maximal). Pulse and blood pressure were monitored during the studies.

We used a battery of cognitive function tests consisting of 4-choice reaction time (13), Stroop word and color-word subtests, (14) and the trail-making B test (15) performed always in this order. These tests were selected because they have been previously used in hypoglycemia research, either singly or as components of a test battery (16). For the 4-choice reaction time (a test of attention, discrimination, and motor speed of reaction), subjects were presented with a computer screen divided into quadrants into one of which a target appeared in random order. Subjects had to respond by pressing the corresponding button on an adapted button box. Targets were presented over a 5-min interval (with a maximum of 500 targets available) with speed and accuracy of response being recorded. The Stroop word and color-word subtests consist of sheets on which are printed the names of colors (red, tan, green, and blue) in incongruously colored ink. In the word subtest, subjects read the words aloud, ignoring the color of the ink. In the color-word (interference) subtest, subjects were asked to identify the color of the ink. Over a 2-min period, 112 words were presented with the time taken for completion (word) and the number of correct responses in 45 s (color-word) recorded. Both Stroop tests require selective attention and mental tracking, with the color-word test additionally requiring color vision and the ability to inhibit conflicting inputs. The Trail-making B test was paper based, with subjects drawing a line connecting a series of alternating numbers and letters (1, A, 2, B, 3, etc.) examining visual, conceptual, and visuomotor tracking. Time to completion was recorded with 4 parallel forms being used to minimize any learning effect. Our cognitive test battery took 10 min to complete and was, therefore, suitable for repeated administration. The whole test battery was practiced on at least 3 occasions on the morning of the study before infusions began and again on 3 occasions during the clamped euglycemic baseline period. Therefore, subjects had completed the test battery on at least 6 occasions before being subjected to a hypoglycemic challenge during HYPO studies to ensure that stable performance was achieved to minimize any practice effects.

Larger aliquots of arterialized blood were collected at times of symptom and cognitive assessment for subsequent assaying of counterregulatory hormone responses. Plasma catecholamines (adrenaline and noradrenaline) were measured using high-performance liquid chromatography (17). Cortisol, free insulin, and glucagon were measured using commercial radioimmunoassay (DPC; Caernarfon, Wales, U.K.). Growth hormone was measured using a commercial immunoradiometric assay (Netria, London). Intra- and interassay variabilities were $<10\%$ for all assays used.

At the completion of the studies, subjects were given a meal and glucose levels were monitored until euglycemia was safely maintained before subjects were allowed to leave.

All results are shown as means ± SEM, unless otherwise stated. Statistical analysis was performed using SPSS for Windows 6.1 (SPSS, Woking, U.K.). Responses to hypoglycemia were initially examined using 2-factor (time and study conditions) repeated measures analysis of variance. Where a significant time by treatment interaction was demonstrated, subsequent nonparametric Wilcoxon's signed-rank test was used to directly compare the EU and HYPO studies to determine the time at which measurements during hypoglycemia first became significantly different from the corre-

Table 1—*Glucose and counterregulatory hormones during EU and HYPO*

			Plateau (min)				Recovery (min)	Analysis of variance		
		Baseline	0	20	40	90	10	Time (*P*)	Study	Time × study
Glucose (mmol/l)	EU	5.2 (0.1)	5.0 (0.1)	5.0 (0.1)	5.1 (0.1)	5.0 (0.1)	5.0 (0.1)	<0.001	<0.001	<0.001
	HYPO	5.2 (0.1)	2.6 (0)*	2.7 (0.1)	2.8 (0.1)	2.6 (0)	6.1 (0.2)			
Epinephrine (nmol/l)	EU	0.3 (0.1)	0.4 (0.1)	0.4 (0.2)	0.4 (0.2)	0.3 (0.2)	0.4 (0.1)	<0.001	<0.001	<0.001
	HYPO	0.2 (0)	0.8 (0.3)	3.1 (0.3)*	4.8 (0.5)	5.7 (0.6)	0.7 (0.1)			
Norepinephrine (nmol/l)	EU	1.5 (0.2)	1.4 (0.2)	1.4 (0.2)	1.5 (0.2)	1.4 (0.2)	1.3 (0.2)	<0.001	<0.01	<0.001
	HYPO	1.8 (0.2)	1.8 (0.2)	2.6 (0.3)*	2.8 (0.2)	2.9 (0.2)	2.5 (0.4)			
Glucagon (ng/l)	EU	24 (19)	26 (16)	17 (12)	24 (14)	13 (9)	—	<0.01	<0.01	0.001
	HYPO	39 (22)	54 (18)	99 (35)*	129 (44)	120 (45)	—			
Cortisol (nmol/l)	EU	267 (36)	276 (16)	261 (19)	260 (25)	231 (29)	—	<0.001	<0.001	<0.001
	HYPO	349 (35)	285 (18)	452 (35)*	608 (29)	719 (24)	—			
Growth hormone (mU/l)	EU	1 (0)	0 (0)	1 (0)	1 (0)	1 (1)	—	<0.001	<0.01	<0.001
	HYPO	1 (0)	0 (0)	12 (4)*	46 (12)	82 (25)	—			

*Threshold (first point at which there was a significant difference between EU and HYPO).

sponding time during the EU studies (i.e., the time of onset of responses).

RESULTS

Insulin, glucose, and pulse/blood pressure

A similar level of hyperinsulinemia was achieved in EU and HYPO (87 ± 5 vs. 94 ± 10, *P* = NS). During EU, glucose levels were maintained at euglycemia throughout (Table 1). During HYPO studies, euglycemia was established for the first 49.2 ± 2.2 min. Plasma glucose was then allowed to fall rapidly over 18.1 ± 1.6 min and this hypoglycemic nadir was then maintained (2.65 ± 0.02 mmol/l) until after completion of the final test battery at 90 min of hypoglycemia. Subsequent euglycemia (target ≥5 mmol/l) was then rapidly reestablished, taking an average of 11.4 ± 1.8 min and maintained until the end of the study (Table 1). Cardiovascular measurements did not change significantly during the EU or HYPO studies (data not shown).

Counterregulatory hormones

Epinephrine, norepinephrine, glucagon, cortisol, and growth hormone all rose during HYPO (Table 1). These hormonal responses were not different from EU when first measured at onset of the hypoglycemic nadir. All of these counterregulatory hormones had risen significantly compared with EU after 20 min of hypoglycemia. There was no trend for hormonal responses to diminish during the sustained 90 min of hypoglycemia. During recovery, sufficient samples were collected for catecholamine assays only. Epinephrine had recovered by 10 min after the restoration of euglycemia but norepinephrine levels remained significantly elevated.

Symptoms

There was no significant change in symptom scores and no one reported feeling abnormal during EU. All subjects became symptomatic during HYPO (Table 2), with significant rises in total, autonomic, and neuroglycopenic scores and with reports of "feeling abnormal" first apparent by 20 min after the onset of hypoglycemia. There was no diminution of symptom scores during sustained hypoglycemia. Immediately after restoration of euglycemia in HYPO studies, 4 of our 8 subjects reported that they felt normal again, with mean feeling abnormal scores for all subjects being statistically indistinguishable from EU studies. By 20 min after restoration of euglycemia, neuroglycopenic symptom scores had recovered. In contrast, autonomic and thus total symptom scores were still significantly reported by subjects 20 min after restoration of euglycemia, although with a clear trend toward recovery.

Cognitive function

During EU, there was no significant change in performance for any of the cognitive tests, indicating that sufficient practice had been performed before the onset of the studies to eliminate any learning effect (Table 2). During HYPO, significant deterioration was seen in the 4-choice reaction time, and in the Stroop word and color-word subtests. In contrast to our findings for counterregulatory hormones and symptoms, all of these measures of cognitive performance had deteriorated significantly when first measured at the onset of the hypoglycemic nadir. The trail-making B test failed to show any deterioration, largely because of the large variability in performance even at euglycemia ($F = 1.2$ time by condition interaction, $P = 0.3$). There was no improvement in any cognitive function test during the sustained 90 min of hypoglycemia. By 20 min after the restoration of euglycemia, subjects had sufficiently recovered so that the scores for the Stroop word and color-word subtests were indistinguishable from those of the EU studies. In contrast, the 4-choice reaction time was still significantly impaired 20 min after blood glucose levels had recovered.

CONCLUSIONS — Our results demonstrate that brain function assessed by sensitive cognitive tests becomes impaired immediately upon reaching significant hypoglycemia. The brain has minimal energy stores so that brain metabolism and thus cognitive function are very dependent on the circulating fuel supply, usually glucose (18).

In contrast to the rapid development of cognitive dysfunction with hypoglycemia, counterregulatory hormone responses took up to 20 min after the onset of the hypoglycemic nadir to be generated. Current thinking is that low glucose levels are predominantly sensed by specialized brain areas, with a fall in the local cerebral metabolism in these regions initiating counterregulatory responses (19,20). In fact, the delay in onset of these responses may be even

Table 2—*Symptoms and cognitive function during EU and HYPO*

			Plateau (min)				Recovery (min)		Analysis of variance		
		Baseline	0	20	40	90	0	20	Time (*P*)	Study	Time × study
Total symptoms	EU	2 (1)	3 (1)	2 (1)	3 (1)	4 (1)	3 (1)	4 (2)	<0.001	0.001	<0.001
	HYPO	3 (1)	4 (1)	10 (2)*	16 (4)	23 (5)	11 (4)	8 (2)			
Autonomic	EU	1 (0)	1 (1)	1 (0)	1 (0)	1 (1)	2 (1)	2 (1)	<0.001	<0.001	<0.001
symptoms	HYPO	1 (0)	2 (1)	6 (1)*	9 (2)	14 (1)	6 (2)	5 (1)			
Neuroglycopenic	EU	2 (1)	2 (1)	1 (1)	2 (1)	3 (1)	2 (1)	2 (1)	<0.001	0.054	0.009
symptoms	HYPO	1 (1)	2 (1)	4 (1)*	7 (2)	9 (3)	5 (2)	3 (1)			
Feeling abnormal	EU	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	<0.001	<0.01	<0.001
score	HYPO	0 (0)	0 (0)	2 (1)*	3 (1)	4 (1)	2 (1)	1 (1)			
Stroop word (s)	EU	49 (3)	48 (2)	50 (3)	51 (4)	50 (3)	50 (2)	48 (3)	<0.001	0.001	<0.001
	HYPO	50 (2)	58 (3)*	59 (3)	60 (4)	65 (5)	56 (2)	53 (3)			
Stroop color	EU	58 (4)	59 (3)	56 (4)	54 (3)	54 (3)	56 (93)	54 (4)	<0.001	0.001	<0.001
word score	HYPO	54 (3)	46 (3)*	46 (4)	44 (4)	43 (4)	49 (3)	50 (5)			
4-Choice	EU	388 (12)	397 (11)	392 (12)	414 (16)	444 (25)	412 (18)	397 (11)	<0.001	0.004	0.011
reaction (ms)	HYPO	422 (23)	477 (29)*	449 (18)	502 (40)	521 (37)	501 (27)	510 (25)			

*Threshold (first point at which there was a significant difference between EU and HYPO).

longer than our results suggest. Neurohumoral responses in healthy volunteers, when assessed by slow-fall hypoglycemic challenges, occur at higher glucose levels than those at which cognitive impairment occurs (typically at ~3.3–3.6 vs. 2.8 mmol/l for cognitive impairment) (5,21), and we did not start measuring until achieving our glucose nadir of 2.6 mmol/l. This delay in onset of counterregulatory hormonal responses may represent central processing delays and/or time for efferent impulses (e.g., increased sympathetic outflow to the adrenal medulla) to trigger downstream effects (e.g., neurosecretory granule exocytosis from the adrenal medulla).

Also in contrast to the effects on cognitive impairment, we found that symptom generation, including the subjective feeling abnormal, was delayed for up to 20 min after the onset of the hypoglycemic nadir and after the onset of detectable cognitive dysfunction. Again, the real delay may have been longer than this because glucose thresholds for the onset of autonomic symptoms assessed by slow-fall hypoglycemic challenges are typically ~3.3 mmol/l (5,6). Autonomic symptoms are believed to relate to neurohumoral activation (12) so that the delay in onset may be anticipated from the delayed onset of this activation. However, the delay in onset of neuroglycopenic symptoms (traditionally ascribed to cortical glucopenia) is less easy to explain in the face of the rapid onset of impaired cognitive performance. There may be direct interaction between autonomic responses and neuroglycopenic symptoms, or cognitive impairment interferes with the ability to recognize neuroglycopenia, or different brain regions are involved. Alternatively, the tests of cognitive function may simply be a more sensitive consequence of brain glucopenia than our ability to detect neuroglycopenic symptoms.

Our failure to find any improvement in cognitive function or diminution in counterregulatory hormones or symptoms during sustained hypoglycemia is consistent with the data from Gold et al. (10), who found no evidence for diminution of responses after 60 min of sustained hypoglycemia. Our data strengthen theirs in that our mini-battery of cognitive function tests was administered repeatedly during a single episode of hypoglycemia, allowing us to follow the temporal changes more precisely. One group has reported a reduction in symptoms and an improvement in cognitive impairment with sustained hypoglycemia (8,9). However, much of this apparent adaptation in symptoms occurred because of a reduction in drowsiness, which may have resulted in an improvement in cognitive performance as a nonspecific effect. An alternative explanation for the improvement in cognitive functioning may have been the higher glucose nadir used (~3 mmol/l in whole blood) (8), which is only just at the reported thresholds in the literature for cognitive dysfunction.

Our results have implications for the design of studies examining hypoglycemia. Most will use a slow-fall hypoglycemic challenge to gradually lower glucose levels in steps of at least 40 min. Our results suggest that this time period is indeed sufficient to allow time for counterregulatory and symptomatic responses to develop so that glucose thresholds for the onset of these physiological responses can be identified. However, a rapid falling hypoglycemic challenge may allow insufficient time so that the glucose level at which responses are detected may not be the level at which these responses were first initiated.

Although neuroglycopenic symptoms diminished immediately on restoration of euglycemia, catecholamine responses were still impaired 10 min after blood glucose recovery, and autonomic and thus total symptoms took up to 20 min to be restored. Despite this, half of our subjects reported that they felt normal again immediately on restoring euglycemia, emphasizing the importance of the neuroglycopenic symptoms in the awareness of hypoglycemia. Delayed recovery of cognitive function after acute hypoglycemia has been reported before, in both healthy and diabetic subjects (22–24), although the finding is not universal (25), possibly because different cognitive function tests have different recovery times (26). The description of subjective recovery before cognitive recovery is new.

In summary, we have demonstrated that during acute hypoglycemia, brain function becomes impaired after glucose levels fall below the threshold for deterioration very rapidly. Protective counterregulatory neurohumoral and symptomatic responses are relatively delayed in onset. We found no adaptation of any responses, cognitive, neurohumoral, or symptomatic,

to sustained hypoglycemia. Indeed, during recovery from hypoglycemia, some aspects of brain function continue to be impaired, even after resolution of symptomatic awareness of hypoglycemia. Our findings have implications for the design of studies examining experimental hypoglycemia. The study needs to be extended to people with diabetes, where discrepancies between subjective awareness of hypoglycemia and its cognitive effects may have important consequences.

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