Brief Report

Awakening and Counterregulatory Response to Hypoglycemia During Early and Late Sleep

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OBJECTIVE—Nocturnal hypoglycemia represents an important problem for diabetic patients, which has been primarily attributed to an attenuated hormonal counterregulation during sleep. So far, hypoglycemia counterregulation has been exclusively examined during early nocturnal sleep, although early sleep differs markedly in sleep stage architecture from late sleep. Here, we investigated whether awakening and counterregulatory responses differ between early and late sleep.

RESEARCH DESIGN AND METHODS—Sixteen healthy subjects were tested on three occasions. On two nights, a linear fall in plasma glucose to a nadir of 2.2 mmol/l within 60 min was induced by insulin infusion. On one night, this was done immediately after sleep onset and on the other night after ~3.5 h of sleep. In a further control night, no hypoglycemia was induced.

RESULTS—During early sleep, 10 subjects awoke in response to hypoglycemia, whereas no subject awoke during the corresponding interval of the control night ($P < 0.004$). During late sleep, all subjects awoke upon hypoglycemia, and four subjects awoke spontaneously during the corresponding control interval ($P < 0.001$). The pattern indicates that the frequency of awakenings caused by hypoglycemia is similar for early and late sleep. Increases in epinephrine, norepinephrine, ACTH, cortisol, and growth hormone were distinctly weaker during late than early hypoglycemia (all $P < 0.05$).

CONCLUSIONS—Diminished hormonal counterregulation during late sleep could be one factor contributing to the clinically observed accumulation of hypoglycemic episodes in the later part of the night in patients with diabetes. ***Diabetes*** **56:1938–1942, 2007**

Hypoglycemic episodes developing during nocturnal sleep represent a major problem in type 1 diabetes (1). Patients with type 1 diabetes frequently fail to awake during nighttime hypoglycemia (2,3). Also, sleep distinctly reduces neuroendocrine counterregulation to hypoglycemia (4,5). So far, studies investigating counterregulatory responses to experimentally induced nocturnal hypoglycemia have focused exclusively on the early part of nocturnal sleep (2–4). However, early and late nocturnal sleep show striking differences in their sleep architecture. Whereas early sleep is dominated by slow wave sleep (SWS), i.e., non-REM (rapid eye movement) sleep stage 3 and 4, in the late part of sleep REM sleep prevails. Notably, the brain's demand for glucose during SWS is overall reduced to a minimum and distinctly lower than in the wake state or during REM sleep (6,7). It could be that the reduced hypoglycemia counterregulation during sleep observed in previous studies results from lowered central nervous demands of glucose, which are particularly low during SWS. Along with the marked differences in sleep stages, secretory activity of most neuroendocrine systems involved in hypoglycemic counterregulation show likewise characteristic temporal dynamics across nighttime sleep (8,9). So far, it is completely unknown whether counterregulation and awakening upon hypoglycemia differs between the early and late part of nocturnal sleep in normal or pathological conditions. Clinically, this question is of particular importance since several studies have reported that ~60–70% of nocturnal hypoglycemic episodes in type 1 diabetic patients occur during late sleep, i.e., between 0300 and 0700 h (10–14).

RESEARCH DESIGN AND METHODS

Sixteen healthy subjects (men/women: 8/8; BMI 23.0 ± 0.6 kg/m^2; aged 28.4 ± 1.5 years) were studied. All subjects had a regular sleep-wake cycle during the 4 weeks before the experiments and had not worked on night shifts. The study was approved by the ethics committee of the University of Lübeck. All subjects gave written informed consent.

Following an adaptation night in the laboratory, each subject was tested on three experimental nights that were spaced at least 2 weeks apart and included standard polysomnography (see online appendix 1 [available at http://dx.doi.org/10.2337/db07-0044]). On two nights, hypoglycemia was induced by intravenous insulin infusion: once the infusion started soon after sleep onset at the first occurrence of stage 2 sleep (early hypoglycemia) and another time after subjects had slept ~3.5 h (late hypoglycemia). In the control night, no hypoglycemia was induced, and no insulin was infused. Experiments were performed in a single-blind fashion, and the order of conditions was assigned according to a within-subject crossover design.

Induction of hypoglycemia followed a standard protocol as previously described in detail (3). The start of insulin infusion varied between 2300 and

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REM, rapid eye movement; SWS, slow wave sleep.

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0045 h in the early hypoglycemia night and between 0245 and 0330 h in the late hypoglycemia night, depending on the exact time the subject fell asleep. Plasma glucose was allowed to fall in a linear manner to a nadir of 2.2 mmol/l, which was reached after 60 min. The temporal dynamics of decreasing glucose concentrations was controlled by simultaneous infusion of a 20% glucose solution whenever necessary. At the nadir concentration of 2.2 mmol/l, insulin infusion was stopped and plasma glucose levels brought back immediately into the normal range by glucose infusion. During the 60-min period of insulin infusion, blood samples were collected every 5 min for determination of counterregulatory hormones. During the remaining night and in the control nights, blood was sampled every 30 min. Blood sampling and infusions were carried out via long thin tubes (dead space 1.5 ml) from an adjacent room without disturbing the subject's sleep. Determination of epinephrine, norepinephrine, ACTH, cortisol, growth hormone, and glucagon was performed as described in online appendix 2.

Statistical analysis. All values are presented as means ± SE. ANOVA was performed on sleep data and on the changes in hormone levels during the hypoglycemic intervals ($value_{end\ of\ interval} - value_{before}$) and during the corresponding control intervals. ANOVA included the repeated-measures factors "hypo" for differences between the hypoglycemic versus control interval (in the control night) and "early/late" for differences between the early and late part of sleep. Additionally, hormonal concentrations during hypoglycemia were analyzed separately, which also accounted for the higher sampling rate during the hypoglycemic than control intervals. The respective ANOVA models included the repeated-measures factors "early/late" for early versus late-night hypoglycemia and "time" for the repeated measurement of the hormones. To detect potential influences of sex and the order of experimental conditions, respective factors were additionally included in the ANOVA models. However, since neither of these factors revealed any significant main or interaction effect, we report here only results from analyses not including the factors sex and order. Pearson correlation analyses were performed to detect potential associations between the extent of counterregulatory hormone responses and the amount of different sleep stages during the early and late hypoglycemic interval. Pairwise comparison for continuous variables relied on Student's *t* test and for categorized variables on the McNemar test. A P value <0.05 was considered significant.

RESULTS

Before the infusion of insulin, plasma glucose concentration was slightly higher during early than late hypoglycemic night (5.8 ± 0.3 vs. 5.1 ± 0.2 mmol/l; $P = 0.029$). The hypoglycemic nadir concentration was identical in both conditions (2.23 ± 0.01 vs. 2.24 ± 0.02 mmol/l; $P = 0.694$). The rate of fall in glucose levels tended to be higher during early than late hypoglycemia (0.057 ± 0.003 vs. 0.048 ± 0.003 $mmol \cdot l^{-1} \cdot min^{-1}$; $P = 0.051$) (Fig. 1). There were no significant differences in serum insulin concentrations (1,014 ± 120 vs. 875 ± 98 pmol/l; $P = 0.602$) and glucose infusion rates (21 ± 5 vs. 16 ± 8 ml/h; $P = 0.257$) during early or late hypoglycemia.

During early hypoglycemia, awakening was observed in 10 of the 16 subjects, whereas during the corresponding time interval of the control night none of the subjects showed polysomnographical signs of awakening ($P = 0.004$). During late-sleep hypoglycemia, all subjects awakened, whereas during the corresponding time in the control night spontaneous awakening occurred in 4 of the 16 subjects ($P < 0.001$). The effect on awakenings caused by hypoglycemia was comparable for early and late sleep because awakenings generally occurred more often during late than early sleep in both the hypoglycemia and the control condition ($\chi^2 = 0.53$, $P > 0.45$ by hierarchical log-linear analysis). Interestingly, none of the subjects awoke from REM sleep. The plasma glucose concentrations at which the subjects awakened were very similar for both hypoglycemic conditions, ranging between 2.2 and 2.7 mmol/l (mean ± SE 2.33 ± 0.05) during early hypoglycemia and between 2.2 and 2.7 mmol/l (2.28 ± 0.05) during late hypoglycemia.

Table 1 summarizes the percent of time spent awake and in the different sleep stages during a 70-min interval comprising the 60 min of insulin infusion and the succeeding 10 min, for early and late hypoglycemic nights and for corresponding time intervals in the control night. Overall, hypoglycemia in comparison with the control-night intervals was associated with an increased time spent awake ($P < 0.001$), with this association not depending on whether hypoglycemia was induced during early or late sleep ($P = 0.717$).

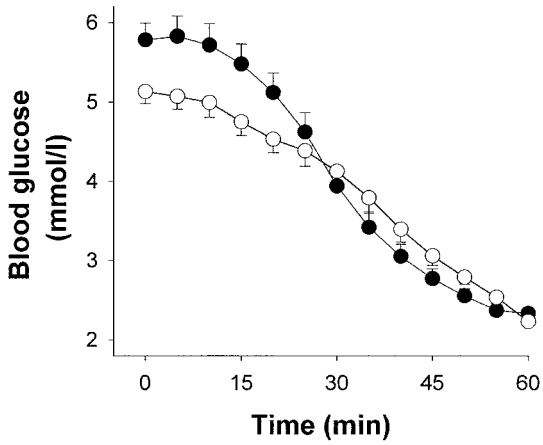

FIG. 1. Means ± SE plasma glucose concentrations during the 60 min of insulin infusion in the early hypoglycemia condition (●) and in the late hypoglycemia condition (○). Insulin infusion started on average at 2336 h (±7 min) during early hypoglycemia and at 0304 h (±3 min) during late hypoglycemia.

Analyses of the changes in counterregulatory hormone levels during the hypoglycemic and corresponding control-night intervals revealed that, except for glucagon, all counterregulatory hormones increased during hypoglycemia, with the rise in epinephrine, ACTH, and cortisol being more pronounced during early than late hypoglycemia (all $P < 0.05$) (Table 2). It should be noted that although peak cortisol levels were similar after early and late hypoglycemia, this peak level only during early sleep reflects an exclusive response to hypoglycemia. During late sleep, levels also increased during the control interval, which indicates that the increase in cortisol upon late hypoglycemia was partly due to the normal circadian rise in cortisol concentration at this time (Table 2).

Separate analyses of the time course of hormonal counterregulation during the two hypoglycemic intervals revealed similar results, indicating a greater increase in epinephrine, norepinephrine, ACTH, cortisol, and also in growth hormone concentrations during early than late hypoglycemia (all $P < 0.05$) (Fig. 2). ANOVA performed on plasma glucagon levels revealed a significant effect of time ($P = 0.043$), indicating an acute decrease in glucagon concentrations in the beginning of the hypoglycemic period, which was followed by a slight increase. However, although glucagon levels tended to be generally higher during early than late hypoglycemia ($P = 0.074$), there was no difference in the temporal course of glucagon concentrations between the two hypoglycemic conditions ($P = 0.498$).

Inspection of individual time courses of plasma epinephrine concentrations revealed that a clear-cut rise in concentrations, exceeding 100% within 5 min, was observed in 12 subjects during early hypoglycemia and also in 12 subjects during late hypoglycemia with 9 subjects showing an increase during both early and late hypoglycemia. Thus, despite the smaller size of the counterregulatory epinephrine

TABLE 1
Percent of time spent awake and in the different sleep stages (S1, S2, SWS, and REM), and percent of epochs containing movements during a 70-min interval including 60 min of insulin infusion and the succeeding 10-min period, for early and late sleep hypoglycemia and for respective intervals in the control nights

	Early sleep		Late sleep		*P*		
	Control night	Hypoglycemia night	Control night	Hypoglycemia night	Early/late	Hypoglycemia	Early/late* hypoglycemia
Wake (%)	0 ± 0	6 ± 3	1 ± 0	6 ± 1	0.981	0.001	0.717
S1 (%)	8 ± 3	12 ± 3	16 ± 6	27 ± 6	0.023	0.053	0.429
S2 (%)	57 ± 6	54 ± 6	55 ± 7	52 ± 6	0.639	0.647	0.982
SWS (%)	33 ± 6	27 ± 7	12 ± 5	8 ± 4	<0.001	0.163	0.893
REM (%)	2 ± 2	0 ± 0	17 ± 4	8 ± 3	<0.001	0.095	0.217
Movement (%)	8 ± 1	19 ± 5	12 ± 3	20 ± 2	0.237	<0.001	0.582

Data are means ± SE. *P* values were derived from ANOVA, including a repeated-measure factor "hypo" for hypoglycemia vs. control night and "early/late" for early vs. late sleep. Early/late* hypoglycemia denotes the interaction term of both factors.

response during late sleep, the number of subjects showing an activation of this neuroendocrine system was not reduced in this late period of sleep. The plasma glucose level at which epinephrine levels started to rise, i.e., the glycemic threshold, was identical during early and late hypoglycemia (2.57 ± 0.14 vs. 2.53 ± 0.09 mmol/l; $P = 0.98$). In all cases, epinephrine levels increased before awakening. However, the time interval between the onset of the epinephrine response and awakening was shorter during early than late hypoglycemia (5.6 ± 1.7 vs. 10.7 ± 2.9 min; $P = 0.050$). Correlation analyses did not reveal any significant association between the amplitude ($value_{end\ of\ interval} - value_{before}$) of counterregulatory hormone responses and the time spent in the different sleep stages during the early and late hypoglycemic interval (all $P > 0.15$).

DISCUSSION

Our data show that in healthy subjects, hormonal counterregulatory responses to hypoglycemia are distinctly

TABLE 2
Serum or plasma concentrations of counterregulatory hormones before and at the end of the hypoglycemic intervals and during the corresponding time interval of the control night in which no hypoglycemia was induced

	First half of night		Second half of night		*P*		
	Hypoglycemia	Control	Hypoglycemia	Control	Early/late	Hypo-gylcemia	Early/late* hypoglycemia
Epinephrine (pmol/l)							
Before	60 ± 5	59 ± 3	62 ± 5	58 ± 2			
End	3,138 ± 928	59 ± 3	1,348 ± 378*	61 ± 4			
Δ	+3,078 ± 927	−1 ± 2	+1,286 ± 378†	+3 ± 3	0.048	0.002	0.048
Norepinephrine (nmol/l)							
Before	0.96 ± 0.10	0.98 ± 0.13	0.71 ± 0.07	0.84 ± 0.08			
End	1.67 ± 0.20	0.82 ± 0.11	1.17 ± 0.12*	0.78 ± 0.09			
Δ	+0.71 ± 0.20	−0.16 ± 0.04	+0.46 ± 0.10	−0.06 ± 0.02	0.364	<0.001	0.162
ACTH (pmol/l)							
Before	1.9 ± 0.2	1.7 ± 0.3	3.3 ± 0.8	1.7 ± 0.2			
End	16.0 ± 5.0	1.4 ± 0.3	8.8 ± 2.1	3.1 ± 0.6			
Δ	+14.1 ± 4.9	−0.3 ± 0.1	+5.5 ± 1.9	+1.4 ± 0.6‡	0.172	0.004	0.045
Cortisol (nmol/l)							
Before	149 ± 28	120 ± 30	164 ± 26	113 ± 26			
End	320 ± 58	92 ± 27	300 ± 44	246 ± 49			
Δ	+171 ± 73	−28 ± 7	+136 ± 40	+133 ± 36*	0.096	0.075	0.025
Growth hormone (μg/l)							
Before	2.6 ± 0.6	3.1 ± 0.8	2.9 ± 1.1	3.8 ± 1.2			
End	8.8 ± 2.3	5.1 ± 1.3	2.7 ± 0.9*	1.7 ± 0.6			
Δ	+6.2 ± 2.2	+2.0 ± 1.6	−0.2 ± 1.1†	−2.1 ± 1.0‡	0.017	0.010	0.205
Glucagon (ng/l)							
Before	159 ± 14	139 ± 14	117 ± 11†	105 ± 9			
End	145 ± 11	128 ± 11	123 ± 9	103 ± 9			
Δ	−14 ± 7	−11 ± 5	+6 ± 7	+2 ± 4	0.084	0.269	0.839

Data are means ± SE. *P* values were derived from ANOVA of Δ values ($value_{end} - value_{before}$) including the repeated-measures factors "early/late" for part of the night (early vs. late) and "hypo" for hypoglycemic vs. control night. Early/late* hypoglycemia denotes the interaction term of both factors. In addition, for pairwise comparisons, Student's *t* test was used, with significant results indicated as follows: *$P < 0.005$ and †$P < 0.05$ for comparison with respective values of the early night; ‡$P < 0.01$ for comparison with the values of the early night control condition.

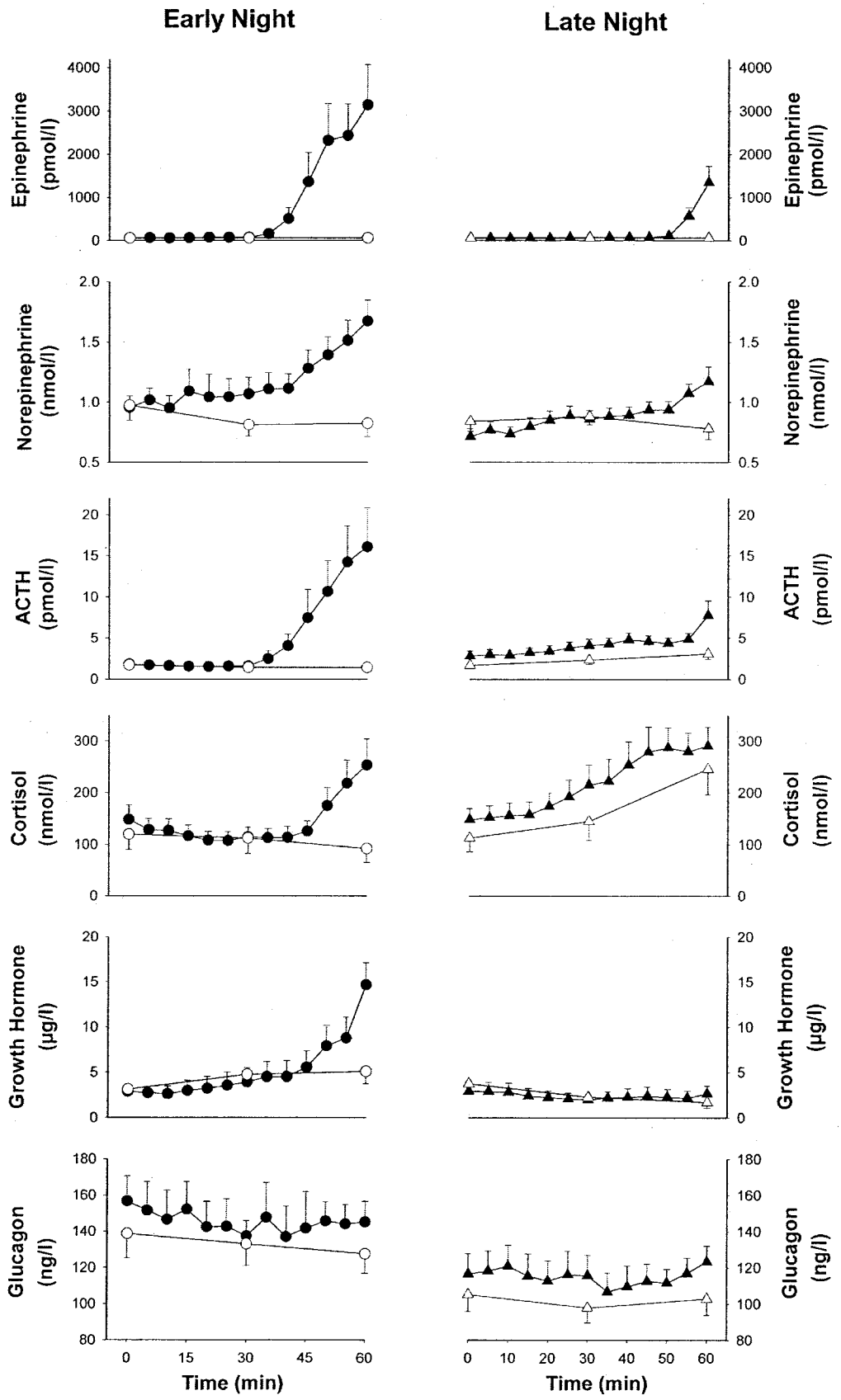

FIG. 2. Means ± SE concentrations of counterregulatory hormones during the 60 min of insulin infusion in early (*left*, ●) and late (*right*, ▲) hypoglycemia nights and during the corresponding time interval in the control night, in which no hypoglycemia was induced (○ and △, respectively).

weaker during the late than early part of nocturnal sleep. While hormonal counterregulation was diminished during late sleep, the arousing effects of hypoglycemia were maintained because the rate of awakenings induced by hypoglycemia during early and late sleep was almost identical.

Based on our data, it cannot be decided whether the decrease in hormonal counterregulation to hypoglycemia during late sleep is an immediate consequence of sleep or caused by circadian oscillators. Previous studies (2,4,5) comparing effects of hypoglycemia during early nocturnal periods of sleep and wakefulness showed that the suppressed hormonal responsiveness to hypoglycemia during early sleep is indeed caused by sleep-dependent mechanisms. Also, hormonal systems involved in hypoglycemia counterregulation (i.e., release of epinephrine and cortisol) are suppressed during REM sleep, which predominates late sleep (9,15,16). This could explain the even more pronounced decrease in hormonal counterregulation observed here during the late phase of nocturnal sleep, although it does not rule out contributions of circadian factors.

Several limitations of the present study need to be mentioned. First, since no hyperinsulinemic-euglycemic clamps were performed during the control night, we cannot fully exclude that the increased number of awakenings during hypoglycemia was facilitated by concurrent hyperinsulinemia. However, previous studies using different modes of insulin and glucose administration have provided evidence that hyperinsulinemia per se does not substantially affect sleep (e.g., refs. 17,18). Second, the higher frequency of blood sampling during the hypoglycemic intervals compared with control intervals could have confounded awakening responses (19). However, blood sampling from an adjacent room was normally not noticed by the subject even when awake. Finally, the rate of fall in glucose levels tended to be higher during early than late hypoglycemia, which may have contributed to the stronger neuroendocrine counterregulatory responses during early sleep.

In light of evidence for an increased frequency of hypoglycemic episodes during late sleep, our finding is of particular clinical relevance. However, it must be pointed out that the present results were obtained in healthy subjects and therefore cannot be directly generalized to patients with diabetes. It will hence be of utmost importance to explore whether similar differences in hormonal counterregulation between early and late nocturnal hypoglycemia likewise exist in diabetic patients.

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