Counterregulatory hormones oscillate during steady-state hypoglycemia

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Genter, Pauline, Nancy Berman, Mary Jacob, and Eli Ipp. Counterregulatory hormones oscillate during steady-state hypoglycemia. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E821–E829, 1998.—During hypoglycemia, the magnitude of the counterregulatory response depends on the extent of plasma glucose reduction. However, our clinical observations during steady-state hypoglycemia indicate that symptom severity can change independently of plasma glucose concentrations, i.e., symptoms appeared to fluctuate despite stable glucose levels. This study was therefore designed to test the hypothesis that hormonal and symptomatic responses to hypoglycemia are pulsatile. Seven healthy subjects had serial blood sampling at 3-min intervals during 90 min of insulin-induced hypoglycemia. Mean ± SE plasma glucose levels plateaued at 62 ± 3 mg/dl. Counterregulatory hormones were significantly elevated (P < 0.05–0.01, except norepinephrine) and strikingly pulsatile. Cluster analysis revealed pulses of large magnitude in plasma glucagon, epinephrine, and norepinephrine concentrations. Amplitudes were, respectively, 72 ± 4, 64 ± 8, and 48 ± 3% of the mean. Interpeak intervals were 27 ± 7, 19 ± 4, and 25 ± 5 min, respectively. Symptom scores and cardiovascular responses were also pulsatile; their peaks were found to coincide with epinephrine peaks. We conclude that hormonal and symptomatic counterregulation in hypoglycemia, while critically driven by plasma glucose levels, is also influenced by an endogenous pulsatility that exists despite steady-state glucose concentrations.

glucose homeostasis; pulsatility; pulse analysis

The defense against hypoglycemia is a fundamental biological protective mechanism. This results from an exclusive dependence of the brain on glucose as a fuel in most normal physiological conditions (7). In the absence of readily available alternative fuels, hypoglycemia can be life threatening if not prevented or rapidly ameliorated. Multiple, redundant mechanisms thus exist to protect against hypoglycemia. The physiology of counterregulation has been carefully investigated; many studies have demonstrated that the acute glucagon and epinephrine responses to hypoglycemia are the most important of the counterregulatory hormonal responses, but they also include the release of growth hormone and cortisol (5, 11, 13, 24, 27, 29–31). The hormonal response drives the liver and kidney to increase endogenous glucose production (EGP) and thereby increase glucose supply to the brain (5, 11, 13, 24, 27, 29–31).

The magnitude of the counterregulatory hormone response to hypoglycemia is dependent on the degree to which plasma glucose concentrations are reduced below normal (24, 31). Once the glucose threshold is exceeded, symptoms and counterregulatory hormones increase with lower glucose levels (24, 31). In recent studies, we have obtained evidence that questions the assumption that symptoms of hypoglycemia are entirely dependent on the prevailing glucose concentration. This is because we observed fluctuation in the symptoms of hypoglycemia despite constant glucose levels (unpublished observations). We therefore hypothesized that, during steady-state hypoglycemia, we would find that the counterregulatory response is pulsatile and that this might explain the intermittent nature of the symptoms we had observed.

However, there is no evidence that any of the counterregulatory hormones oscillate during hypoglycemia. Studies during nonhypoglycemic conditions have demonstrated that some counterregulatory hormones are released in pulsatile fashion, but most are secreted with a frequency that is circadian (cortisol) or ultradian (growth hormone, catecholamines; see Refs. 26, 34, 35). This relatively low frequency inherent in counterregulatory hormone pulsatility is not consistent with its potential life-sustaining role during hypoglycemia, where rapid responsiveness would be desirable. Glucagon is the only hormone of this group that is known to oscillate in human subjects within a time frame that will permit a physiological role during acute counterregulation, i.e., with a period of ∼10–25 min (14, 15, 20). Although glucagon might also oscillate during hypoglycemia, this is currently unknown. Oscillations of epinephrine, the other major counterregulatory hormone response during acute hypoglycemia, have not been studied in human subjects. This is because basal plasma epinephrine concentrations are at or below the sensitivity limit of most assays for this hormone; thus analysis of potential oscillations is not possible. In the present study, using a model of ongoing, stable hypoglycemia in normal human subjects, we provide evidence for the occurrence of pulsatility in certain counterregulatory hormones, unrelated to changes in glycemia. These findings suggest that hypoglycemic counterregulation, like many other biological systems, is inherently pulsatile and occurs with a frequency that is compatible with an important biological role.

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METHODS

Subjects

Seven healthy adult volunteers (5 women and 2 men; mean age 30 ± 4 yr) participated in the study. The subjects were of normal weight (mean body mass index 21.7 ± 2.8 kg/m²), none were using any medications including oral contraceptives, and there was no personal or immediate family history of diabetes or glucose intolerance. All had consumed a weight-maintaining diet, which included at least 200 g carbohydrate daily, for 3 days before the study. The experiments were conducted in the Clinical Research Center at Harbor-UCLA Medical Center (Torrance, CA). Prior informed consent was obtained, and the institution’s human subjects review committee approved all studies.

Experimental Design

Each subject participated in the following two separate experiments, at least 1 wk apart: 1) in the basal state (saline infusion) and 2) during insulin-induced mild hypoglycemia. Saline infusion was specifically chosen as the control rather than euglycemic glucose clamp because glucose clamps have an exogenous oscillatory rhythm induced by the feedback process inherent in the method (e.g., see Ref. 4). We wished to exclude exogenous oscillatory factors that might influence the spontaneous endogenous rhythms that we were testing. All experiments were conducted in the morning after a 10-h overnight fast. The subjects assumed a resting recumbent position, and a catheter was inserted in an antecubital vein for infusion of saline or insulin. A second catheter was placed in retrograde fashion in a dorsal hand vein of the opposite arm for blood sampling, and the hand was kept warm with a heating pad for arterIALIZation of venous blood (19). Subjects rested for 60 min before the start of the infusion period. Either saline or regular human insulin (Eli Lilly, Indianapolis, IN) was infused via an IMED pump for 180 min. Insulin was infused at a rate of 40 mU·kg⁻¹·h⁻¹, a rate that has been shown to induce mild hypoglycemia (mean ~60 mg/dl) in normal subjects (19).

Arterialized blood samples were drawn for determination of baseline plasma glucose concentrations before the start of the infusion period and at 20-min intervals over the first 80 min of the infusion period. From 90 min onward, 30 samples were drawn at precise 3-min intervals over the final 90 min of the infusion period for determination of plasma glucose, glucagon, growth hormone, cortisol, epinephrine, and norepinephrine concentrations. Pulse, blood pressure, and symptom score were also monitored at the bedside at 3-min intervals over the last 90 min of the insulin infusion. For safety purposes, serum glucose concentrations (Yellow Springs Instruments) were also monitored at the bedside.

Analytical Methods

Blood was collected in cooled tubes and maintained at 4°C until centrifugation, which was carried out within 60 min. Plasma was frozen at −70°C before analysis. All intra-assay coefficients of variation (CV) for hormone assays were performed on 10 replicate samples; and inter-assay CV was performed on at least ten samples. Inter-assay CV values were evaluated at 6-mo intervals, and all CV reported here were performed throughout the time that these studies were carried out. All samples, known and unknown, were assayed in duplicate. The samples from each individual study were always placed in a single assay for each of the hormones measured. Samples were assayed for plasma glucose with a modified hexokinase/glucose dehydrogenase method using an Abbott Bichromatic Analyzer (ABA-1). Both the intra- and inter-assay CV were 1.8% at mean glucose concentrations of 82 (n = 5) and 286 (n = 10) mg/dl. Plasma glucagon immunoreactivity was determined using a previously described (12) double-antibody radioimmunoassay (RIA). Intra-assay CV was 8.8 and 5.6% at mean glucagon concentrations of 77 and 244 pg/ml, respectively. Inter-assay CV was 13.0% at a mean glucagon concentration of 274 mg/dl; lower limit of sensitivity was 25 pg/ml (12).

Plasma growth hormone and cortisol were measured using previously described RIA techniques (23). The intra-assay CV for growth hormone was 10.4 and 4.0% at mean growth hormone concentrations of 1.7 and 8.2 ng/ml, respectively. Inter-assay CV was 10.8% at mean growth hormone concentration of 7.4 ng/ml; the lower limit of sensitivity of the assay was 0.25 ng/ml. The intra-assay CV for cortisol was 5.2 and 4.0% at mean cortisol concentrations of 5.5 and 10.8 ng/ml, respectively. Inter-assay CV was 8.2% at mean cortisol concentration of 14.8 ng/ml; the lower limit of sensitivity was 2 ng/ml. Epinephrine and norepinephrine in plasma were measured using a radioenzymatic assay as previously described (17), each with a lower sensitivity limit of 20 pg/ml. The intra-assay CV for epinephrine was 12.3 and 8.3% at mean epinephrine concentrations of 30 and 71 pg/ml, respectively. Interassay CV was 16% at a mean epinephrine concentration of 46 pg/ml. The intra-assay CV for norepinephrine was 14.6 and 8.4% at mean norepinephrine concentrations of 26 and 51 pg/ml, respectively. The interassay CV was 14.0% at a mean norepinephrine concentration of 108 pg/ml.

Symptoms were collected using a modification of a previously devised questionnaire (17). Nine different symptoms were evaluated on a scale of 0 to 10. The scores for each symptom at each time point were added together to provide a composite score, presented in arbitrary units. The symptoms evaluated were weakness, sweating, heart pounding, hunger, headache, dizziness, shakiness, nervousness, and sleepiness. Subjects were questioned every 3 min in both studies. The studies (insulin or saline infusion) were administered in random fashion without the subject being informed of the order, in order not to influence symptoms. Five of seven subjects became slightly symptomatic during the insulin infusion; however, blood pressure and pulse remained relatively stable throughout the test period, and all experiments were completed uneventfully.

Only two of the symptoms appeared during the saline infusion, hunger and sleepiness. The other seven symptoms were uniformly scored as zero during the saline infusion. Furthermore, all five subjects who became symptomatic reported these symptoms. These seven symptoms are therefore presented in this study. For the purposes of this study, we regard these symptoms as specific for hypoglycemia.

Statistical Analysis

The time-series data (samples collected at 3-min intervals for 90 min) from each experiment were analyzed to determine their patterns of variation over time using the cluster pulse detection program (37). The time series may either 1) be constant with some noise but no detectable variation above the noise level (steady state) or 2) show sharp increases over a baseline followed by decreases back to the baseline level (pulses) at intervals that may or may not be regular. A steady-state condition will be identified when no pulses are detected. The difference between the nadir and the peak of a pulse constitutes the peak amplitude and is expressed in terms of the mean plasma concentration (%mean) of plasma
glucose and hormone concentrations or symptom score. Mean plasma glucose and hormone concentrations during the 90-min frequent sampling period of hypoglycemia and control experiments were compared using the Student's t-test for paired samples. Significance levels were set at P < 0.05. Results are presented as means ± SE unless otherwise indicated.

RESULTS

Mean Group Response to Insulin-Induced, Mild Hypoglycemia

The induction of mild hypoglycemia (plasma glucose plateau >50 and <70 mg/dl) was achieved during insulin infusion in all subjects (n = 7). Mean ± SE plasma glucose concentrations fell from 98.9 ± 2.3 mg/dl at baseline to 63.3 ± 2.8 mg/dl by 90 min. They remained at a mean plateau level of 62 ± 3 mg/dl during the 90-min frequent sampling period (Fig. 1 and Table 1). A striking hormonal response to low plasma glucose levels was demonstrated in all studies (Table 1). The mean change in plasma glucagon concentration was significantly greater during the hypoglycemia study compared with the same 90-min period in the basal study, 233 ± 17 vs. 87 ± 3.7%, respectively (expressed as %baseline, P < 0.02). Mean plasma epinephrine during hypoglycemia was increased compared with the euglycemic study, 340 ± 75 vs. 71 ± 29 pg/ml (P < 0.01); mean plasma growth hormone was similarly elevated, 25 ± 5.7 vs. 1.1 ± 0.4 ng/ml (P < 0.01), as were mean cortisol concentrations, 17 ± 1.5 vs. 6 ± 0.8 µg/ml (P < 0.01). Mean plasma norepinephrine (251 ± 22 pg/ml) levels were also increased during hypoglycemia compared with the basal study (206 ± 22 pg/ml), but the difference was not statistically significant.

When plasma glucose and hormone concentrations are presented in conventional fashion as the mean ± SE of the group, using only those blood samples obtained at 15-min intervals, the data appear as illustrated in Fig. 1. In this figure, the effects of constant insulin or saline infusion between 90 and 180 min are shown (for glucose, data for the first 90 min are also included). When presented in this manner, these data demonstrate little, if any, pulsatile fluctuation in either experiment. These results are similar to findings in previous studies using this or a similar sampling frequency and insulin infusion rate (17, 29–31).

![Fig. 1. Effect of insulin (●) or saline (△) infusion on mean plasma glucose and counterregulatory hormone concentrations in normal subjects (15-min sampling intervals; n = 7 subjects).](http://ajpendo.physiology.org/Downloaded from http://ajpendo.physiology.org/)
During insulin infusion, plasma glucose concentrations remained remarkably stable (CV = 2–4%). In spite of this, the variation in CV for certain counterregulatory hormones was considerably larger. The mean CV for glucagon (31 ± 6%), epinephrine (27 ± 2%), growth hormone (24 ± 6.3%), and norepinephrine (19 ± 1%) was relatively large, whereas the CV for cortisol was only 12 ± 2% (Table 1). The CV of glucose and the counterregulatory hormones were not significantly different during hypoglycemic and euglycemic states. The large CV in some of the counterregulatory hormones, in the presence of high mean plasma concentrations observed during hypoglycemia (Table 1), suggested that analysis of individual responses might provide evidence of large fluctuations in those hormone concentrations.

Individual Responses of Plasma Glucose and Hormones During Hypoglycemia

Plasma glucose and hormone concentrations during individual insulin infusion experiments were therefore evaluated using all of the samples obtained (n = 30), during frequent serial sampling at 3-min intervals. The results from a single subject are illustrated in Fig. 2. Analysis of this individual’s data with the Cluster program revealed consistent, high-amplitude peaks in most of the counterregulatory hormones. The amplitudes of these peaks (expressed as percent of the mean) for plasma glucagon, epinephrine, and norepinephrine concentrations were 82, 81, and 58%, respectively, in this subject. In contrast, this subject had only a few low-amplitude peaks (mean = 9%) in the plasma glucose concentration, and no peaks were identified for plasma growth hormone or cortisol. Results of peak identification analysis during hypoglycemia for all seven subjects are shown in Table 2. The results of the other six subjects are similar to the observations in the single subject shown in Fig. 2.

<table>
<thead>
<tr>
<th></th>
<th>Basal State</th>
<th>Hypoglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>CV, %</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>100 ± 2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Cortisol, µg/ml</td>
<td>6 ± 1</td>
<td>14 ± 2.3</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>206 ± 22</td>
<td>22 ± 7.3</td>
</tr>
<tr>
<td>Growth hormone, ng/ml</td>
<td>1 ± 1</td>
<td>48 ± 14</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>71 ± 46</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>127 ± 49</td>
<td>33 ± 5</td>
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</tbody>
</table>

Values are means ± SE; n = 7 subjects except for glucagon where n = 6 subjects (data unavailable for 1 subject). The 90-min sampling period was the period between 91 and 180 min after initiation of insulin infusion. CV, coefficient of variation. *Significantly different from basal study, P < 0.01; †significantly different from basal study, P < 0.05.

During insulin infusion, plasma glucose concentrations remained remarkably stable (CV = 2–4%). In spite of this, the variation in CV for certain counterregulatory hormones was considerably larger. The mean CV for glucagon (31 ± 4%), epinephrine (27 ± 2%), growth hormone (24 ± 6.3%), and norepinephrine (19 ± 1%) was relatively large, whereas the CV for cortisol was only 12 ± 2% (Table 1). The CV of glucose and the counterregulatory hormones were not significantly different during hypoglycemic and euglycemic states. The large CV in some of the counterregulatory hormones, in the presence of high mean plasma concentrations observed during hypoglycemia (Table 1), suggested that analysis of individual responses might provide evidence of large fluctuations in those hormone concentrations.

![Fig. 2. Fluctuations in plasma glucose and counterregulatory hormones during insulin-induced hypoglycemia using 3-min sampling intervals (n = 1).](http://ajpendo.physiology.org/)
Table 2. Peaks in plasma glucose and counterregulatory hormone concentrations in healthy subjects during mild hypoglycemia using cluster analysis program

<table>
<thead>
<tr>
<th>Cluster Analysis</th>
<th>Glucose</th>
<th>n</th>
<th>No. of peaks</th>
<th>Amplitude (%mean)</th>
<th>Interval, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>6/7</td>
<td>1.8 ± 0.8</td>
<td>9 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucagon</td>
<td>6/6</td>
<td>3.0 ± 0.5</td>
<td>73 ± 4</td>
<td>27 ± 7</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>7/7</td>
<td>3.4 ± 0.4</td>
<td>64 ± 8</td>
<td>19 ± 4</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine</td>
<td>7/7</td>
<td>2.7 ± 0.4</td>
<td>48 ± 3</td>
<td>25 ± 2</td>
</tr>
<tr>
<td></td>
<td>Growth hormone</td>
<td>4/7</td>
<td>2.3 ± 0.5</td>
<td>65 ± 12</td>
<td>29 ± 9</td>
</tr>
<tr>
<td></td>
<td>Cortisol</td>
<td>3/7</td>
<td>1.3 ± 0.4</td>
<td>29 ± 6</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of subjects in which peaks were detected/number of subjects analyzed. Interval, interpeak interval.

Peaks were observed for each hormone in each subject. Peak amplitudes (%mean) were 73 ± 4, 64 ± 8, and 48 ± 3%, respectively, for glucagon, epinephrine, and norepinephrine concentrations during the hypoglycemia experiments (Table 2). The estimated interpeak interval was 27 ± 7, 19 ± 4, and 25 ± 2 min, respectively. Pulsatility in the case of growth hormone was inconsistent; peaks were observed in only four of seven subjects. These four subjects demonstrated high-amplitude (mean = 65 ± 24%) peaks in plasma growth hormone concentrations during the hypoglycemia experiment (Table 2). Interpeak interval was 29 ± 9 min, similar to that found for the other hormones. Plasma cortisol concentrations were not convincingly pulsatile; a few low-amplitude (mean = 29 ± 6%) pulses were identified in only three subjects only (Table 2). Too few peaks were found to calculate a mean interpeak interval for cortisol.

Euglycemic studies. During euglycemia, plasma glucose concentrations showed few peaks (Table 3). In evaluation of pulsatility of the hormones, basal concentrations in some cases were below the assay sensitivity so that analysis was not possible. In these basal experiments, peaks in plasma glucagon concentrations were identified for all subjects. The estimated interpeak interval was 27 ± 7 min, and peak amplitude was 73 ± 14% (Table 3). For the other hormones, few subjects demonstrated pulsatility, and the amplitude of those peaks was three- to fivefold lower than during hypoglycemia. Only three subjects had basal plasma epinephrine concentrations consistently greater than the sensitivity of the assay (<20 pg/ml) that could be analyzed (Table 3). Consistent peaks were not identified in the majority of subjects for plasma norepinephrine, growth hormone, or cortisol during the basal experiment in the time period tested (Table 3). Four subjects had basal growth hormone concentrations less than the lower limit of the assay sensitivity (<0.25 ng/ml); therefore, these data were also not analyzed for peaks.

Fluctuation of Symptoms and Cardiovascular Variables During Hypoglycemia in Individual Subjects

Overall, cardiovascular changes and symptoms developed by the subjects were mild (e.g., Fig. 3); the maximum symptom score experienced at any one time was 14 arbitrary units, out of a possible total score of 70 units, except in one subject whose maximal score was 32. As demonstrated in Fig. 3C, in data from the same subject shown in Fig. 2, the total symptom score was also pulsatile. In addition, peaks were observed in systolic blood pressure (Fig. 3D) and pulse rate (Fig. 3B). All peaks in the three physiological variables were identified by the cluster program. The relationship between the physiological variables and plasma epinephrine concentrations is illustrated in Fig. 3. This figure demonstrates that the peaks in epinephrine and the physiological responses all occurred at about the same time points, i.e., on three occasions in this subject.

Analysis of the other subjects revealed an overall high coincidence between peaks of epinephrine and peaks in the physiological variables. A total of 15 epinephrine peaks were identified in five subjects (Table 4). Peaks in pulse rate coincided with 14 of the 15 epinephrine peaks (Table 4). Peaks in systolic blood pressure coincided with all of the epinephrine peaks (7 of 7 subjects in whom data were available). Peaks in symptom score coincided in 9 of 13 epinephrine peaks observed in symptomatic subjects. In total, if one assumes that each peak of epinephrine (n = 15) coincides with a peak in each of the three physiological measures (symptoms score, systolic blood pressure, pulse rate), then there would be 45 possible pairs of coincident peaks. Some data were unavailable for 10 of these pairs (see Table 4), so that 35 potential coincident pairs remain. The data show that there were 30 coincident pairs out of a possible total of 35. This is a coincidence rate of better than 85% between epinephrine peaks and the three associated physiological variables.

DISCUSSION

The counterregulatory response to hypoglycemia has been well characterized in recent years (for reviews, see Refs. 8 and 9). Therefore, the hormonal and metabolic changes during acute lowering of plasma glucose levels...
are well understood. Most of those studies were carried out using transient hypoglycemia after bolus injection of insulin or stepwise reduction of plasma glucose concentrations as the predominant models. In neither case did the plasma glucose remain at a plateau for very long before glucose levels changed. However, in recent studies using a model of prolonged, continuous mild hypoglycemia (12, 18, 19), we made two contrasting observations. 1) During continuous insulin infusion at rates that did not overwhelm glucose homeostasis, normal human subjects were able to maintain a fairly predictable plateau of circulating glucose concentrations for periods as long as 200 min. This suggested a steady state arising from a balance of continuous insulin infusion and a stable counterregulatory response. 2) In contrast, clinical responses observed at the bedside did not fit this model. Despite stable glucose concentrations, hypoglycemic symptoms were intermittent in nature. Because oscillatory phenomena are common in other biological models, including the physiology of glucose metabolism (1–3), we reasoned that the counterregulatory hormone response during hypoglycemia also might not be in steady state, despite stable glucose concentrations, i.e., the hypoglycemic response might be characterized by intermittent pulses or peaks of counterregulatory hormones. We therefore hypothesized that, during hypoglycemia, we would be able to document intermittent symptoms and that these would be associated with fluctuations in the counterregulatory response, in particular manifesting as pulsatility of plasma catecholamine concentrations.

The results of this study confirmed the hypothesis in a number of respects. First, the data unequivocally demonstrated the presence of pulsatility in counterregulatory hormones during hypoglycemia in human subjects. Indeed, this is the first demonstration of pulsatility of plasma epinephrine or norepinephrine concentrations in human subjects and of glucagon during hypoglycemia. Second, high-amplitude pulses were observed during hypoglycemia. Pulses of largest amplitude were observed in those hormones that play the most critical role in the counterregulatory response to acute hypoglycemia, i.e., glucagon and epinephrine. Third, pulses occurred with a mean interpeak interval of 30 min. The frequency of these pulses is consistent with a biological role for counterregulatory hormones during short-term hypoglycemia, because they are of higher frequency than previously described ultradian or diurnal rhythms described in nonhypoglycemic situations (16, 26, 34, 35). Fourth, pulsatility was observed in symptom score as well as in the cardiovascular physiological variables measured. Pulse rate, systolic blood pressure, and symptom score fluctuated in concert. In most cases these peaks tended to coincide with peaks in plasma epinephrine concentrations, suggesting, but not proving, a possible cause-effect relationship.

Table 4. Number of coincident peaks: epinephrine and physiological measures

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Epinephrine Peaks</th>
<th>Pulse*</th>
<th>Systolic BP*</th>
<th>Symptoms*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
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<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>No Sx</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>ID</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>4</td>
<td>ID</td>
<td>2</td>
</tr>
</tbody>
</table>

Peaks were detected in the time-series data using the cluster program. *Number of peaks in each of the physiological measures that coincided with epinephrine peaks; all peaks identified by the cluster program. BP, blood pressure; ID, incomplete data; No Sx, no symptoms.
Although a few epinephrine peaks did not coincide with symptom score, this discrepancy was accounted for by two subjects in whom fewer peaks in symptom score were found compared with epinephrine (Table 4, subjects 4 and 5). In each case, plasma epinephrine concentrations were low when symptom peaks were missing, below the threshold required to provoke a symptomatic response (6). This explains the lack of symptoms at those times. In the same two subjects, epinephrine peaks did coincide with pulse rate peaks, confirming the physiological relevance of the epinephrine pulses, despite absence of symptoms. Thus the findings of pulsatility of both counterregulatory hormones and physiological variables during hypoglycemia supported the hypothesis and confirmed our initial clinical observations.

The high sampling frequency used in this study allowed us to evaluate more closely the possibility of pulsatility in plasma glucose concentrations, which may have been missed in previous studies when blood samples were obtained using routine sampling intervals at 10- to 20-min intervals. The data demonstrate that plasma glucose concentrations were indeed stable, with very few low-amplitude peaks. The CV of plasma glucose concentrations during insulin-induced hypoglycemia in this study (i.e., 2–4%) was similar to that described in experimental euglycemic or hyperglycemic glucose clamps (e.g., see Ref. 10). In the current study, an “endogenous” glucose clamp at hypoglycemic levels was induced, without the need for exogenous glucose infusion. Still, glucose levels nevertheless remained at steady state once hypoglycemia was attained. This attests to a remarkable degree of fine tuning of the glucose homeostatic system, considering the large, concomitant pulsatility of counterregulatory hormones demonstrated in this study. In each case, the amplitude of the peaks and the CV of plasma hormone concentrations were greatly above the CV for their respective assays. These fluctuations thus cannot be explained by assay variation.

In the nonhypoglycemic state, pulsatility of hormones involved in glucose regulation is well recognized. Fasting plasma insulin and glucagon concentrations oscillate in a rapid, sustained manner with similar frequencies (9–14 min) in nondiabetic humans, dogs, baboons, monkeys, and rats under basal conditions (4, 14, 15, 20, 22). Other classic fuel-regulating hormones such as growth hormone, cortisol, and catecholamines were also reported to be pulsatile in several animal models under fasted, basal conditions, demonstrating pulsatility of the ultradian or diurnal type (16, 26, 34, 35). Higher-frequency fluctuations of these hormones, such as observed in this study, have not previously been examined in humans. Furthermore, to our knowledge, no studies of pulsatility of any of these hormones during hypoglycemia have previously been reported.

The mechanism for pulsatility of counterregulatory hormones during hypoglycemia is unknown. However, these studies raise the possibility that our findings in hypoglycemia may be an amplification of a preexisting pulsatility observed in the basal state. Although the finding of glucagon pulsatility in the basal state is not new (14, 15, 20), as pointed out above, high-frequency epinephrine pulsatility has not been described previously in human subjects. Our data strongly suggest that epinephrine does oscillate in the basal state, provided plasma concentrations are high enough to be measured. This needs to be confirmed with a larger number of subjects who have plasma levels in the measurable range. Pulsatility observed in the basal state suggests that the pulsatile process may be inherent in the secretion of certain counterregulatory hormones. If so, reducing plasma glucose may induce large hormonal pulses not as a de novo phenomenon but by enhancing the magnitude of basal pulses. The finding of epinephrine pulses during euglycemia also raises the question of whether insulin oscillations could be a reflection of catecholamine pulsatility in the basal state. This seems unlikely, however. Plasma epinephrine concentrations during euglycemia are probably too low to influence insulin secretion (6), and insulin oscillations are observed in the denervated state (2), suggesting that norepinephrine also does not play a role in initiating basal insulin oscillations.

The results of this study may provide an explanation for the apparent discrepancy in the response of EGP to the effects of counterregulatory hormones when these are increased by continuous hypoglycemia vs. the effects of exogenous infusion of the same hormones. When insulin is infused continuously to induce and maintain hypoglycemia, mean counterregulatory hormones and endogenous production of glucose are increased, and both are maintained at an elevated level throughout (29). However, when either glucagon (32, 33) or epinephrine (32, 36) or both together (23) are infused continuously to similar levels, EGP increases only transiently, returning to baseline despite continued hormone infusion. This suggests that endogenous hormone secretion is more effective than exogenous infusion to stimulate glucose production. Pulsatility of the endogenous counterregulatory response to hypoglycemia may explain this discrepancy because considerable evidence in other systems suggests that pulsatile hormone delivery is more biologically effective than continuous infusion (28). Although this study cannot address the biological efficacy of pulsatility in the counterregulatory hormones, others have reported that glucagon is more effective to stimulate hepatic glucose output in vitro and in vivo when delivered in pulsatile fashion (21, 25, 38). Continuous delivery of glucagon and epinephrine is associated with downregulation of biological activity, as manifested by their effects on EGP (6, 33, 36); thus intermittent or pulsatile secretion may provide the ongoing counterregulatory effect required to prevent hypoglycemia during insulin infusion, as in this experimental model.

An important part of the counterregulatory process may therefore depend on appropriate pulsatility in glucagon and epinephrine concentrations during hypoglycemia. This is supported by the finding that the very hormones that play the most important role in counterregulation during hypoglycemia (glucagon and epineph-
line) were also found to have the greatest pulsatility in these studies. We conclude that pulsatility is an inherent response of the counterregulatory process in hypoglycemia, possibly resulting from amplification of basal oscillations when plasma glucose is reduced. Furthermore, the frequency of pulses is consistent with the biological role of counterregulatory hormones. We therefore speculate that hormonal pulses may be of importance for optimal biological action in this setting. Finally, there is also potential clinical relevance to these findings; initial relief of symptoms during hypoglycemia may not necessarily reflect improvement in plasma glucose concentrations. It may only be a manifestation of a spontaneous nadir in the pulsatile counterregulatory hormone response.

We are indebted to the nurses, dietary staff, and core laboratory technicians of the Clinical Study Center at Harbor-University of California Los Angeles Medical Center for excellent assistance in the performance of these studies. These studies were supported in part by a grant from the American Diabetes Association and Division of Research Resources Grant M01-RR-00425.

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Received 23 April 1998; accepted in final form 23 July 1998.

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