Increased high-frequency heart rate variability during insulin-induced hypoglycaemia in healthy humans

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ABSTRACT

Despite causing sympathetic activation, prolonged hypoglycaemia produces little change in HR (heart rate) in healthy young adults. One explanation could be concurrent parasympathetic activation, resulting in unchanged net effects of autonomic influences. In the present study, hypoglycaemic (2.7 mmol/l) and normoglycaemic (4.7 mmol/l) hyperinsulinaemic clamp studies were performed after normoglycaemic baseline clamp periods with 15 healthy volunteers (seven male; mean age, 27 years) on two occasions in a randomized single-blind cross-over design. Non-invasive indices of cardiac autonomic activity and hormones were measured at baseline and 1 h after the beginning of hypoglycaemia or control normoglycaemia. Plasma insulin levels and mean HR were similar during both conditions. During hypoglycaemia, there was a 485 % increase in plasma adrenaline (epinephrine). A shortening of the pre-ejection period by 45 % suggested strong sympathetic cardiac activation. High-frequency (0.15–0.45 Hz) HRV (HR variability) increased, indicating a concomitant increase in parasympathetic tone. Thus, during hypoglycaemia-induced sympathetic cardiac activation in healthy adults, parasympathetic mechanisms are involved in stabilizing mean HR.

INTRODUCTION

The sympathetic counter-regulatory response to hypoglycaemia is characterized by systemic adrenaline (epinephrine) and noradrenaline release [1] and enhanced sympathetic neural activity [2,3]. It is assumed that such sympathetic activation induces tachycardia [4]; however, several early studies described healthy individuals displaying a different response pattern of stable HR (heart rate), despite clear hypoglycaemia [5], or a return of HR to baseline levels, despite continuing increases in plasma adrenaline concentration [6]. Recent studies (i.e. [7,8]) confirm these early reports in that minimal non-significant HR changes were observed during hypoglycaemia, despite the presence of the classical sympathetic counter-regulatory response that is known to involve cardiac sympathetic activation [9]. Such findings could be explained by increased vagal transmission to the heart preventing increases in HR, but available reports are controversial, describing hypoglycaemia-induced parasympathetic augmentation leading to atropine-sensitive sinus bradycardia [10], and hypoglycaemia-induced parasympathetic withdrawal leading to tachycardia in sympathectomized humans [11]. As there is no direct

Key words: autonomic nervous system, glucose, heart rate variability, hypoglycaemia, impedance cardiography.

Abbreviations: HF, high frequency; HR, heart rate; HRV, HR variability; IBI, interbeat intervals; i.v., intravenous; LVET, left ventricular ejection time; PEP, pre-ejection period; RMSSD, root mean square of successive differences.

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method to assess parasympathetic cardiac activity, studies of HF (high-frequency) HRV (HR variability) can provide further information on this issue; however, the two available studies report conflicting results: a case report [12] indicated increased HRV during hypoglycaemia, whereas a recent study [8] suggested no change in HRV. The aim of the present study was to clarify this issue and examined parasympathetic HR control by spectral analysis of HR time series, which were obtained in the presence of cardiac sympathetic activation triggered by experimental hypoglycaemia of extended (1 h) duration. Time-domain-based indices of HRV, such as S.D. and RMSSD (root mean square of successive differences) of IBI (interbeat intervals) [13,14] were also used, because they are simple and readily understood. Time domain measures of parasympathetic cardiac control [15] that are rather resistant to the effects of changing breathing patterns [14].

It was hypothesized that parasympathetic cardiac influences would increase during hypoglycaemia, as reflected by increased HF HRV. Furthermore, it was hypothesized that sympathetically activated cardiac influences (either affected neurally or via systemic adrenaline) would increase during hypoglycaemia, as reflected by shortening of the cardiac PEP (pre-ejection period). Plasma catecholamines and glucagon were examined to demonstrate the initiation of normal counter-regulatory responses to hypoglycaemia. Because hyperinsulinaemia activates the sympathetic nervous system [16] and interacts with the parasympathetic nervous system [17], similar plasma insulin levels were implemented during target hypoglycaemia and target normoglycaemia.

METHODS

Subjects
Fifteen healthy volunteers (seven male; mean age, 27 years) with normal findings on physical examination, routine blood chemistry and haematology and standard electrocardiography participated in the study. Exclusion criteria were current smoking or evidence of any illicit drug or sedative use. Subjects were asked to refrain from alcohol, caffeine and food for 12 h before the laboratory sessions. Subjects read and signed an Institutional Review Board-approved informed consent form prior to participation. The research was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Basel University Hospital.

Experimental procedures
Normo- and hypo-glycaemic clamp studies were performed at the Basel University Hospital 4 weeks apart, at 08:00 hours, according to a randomized single-blinded cross-over design. An i.v. (intravenous) catheter was inserted into a dorsal vein of the left hand, which was placed in a heated box (52°C) to arterialize the venous sample. Another i.v. catheter was inserted into a cubital vein of the same arm for glucose and insulin infusion. A loading dose of human insulin (0.01 unit/kg of body weight; Novo Nordisk, Mainz, Germany) was given, followed by continuous insulin infusion (1 milliunit·min⁻¹·kg⁻¹ of body weight). Glucose infusion (20 % dextrose solution) was started at 2.5 mg·min⁻¹·kg⁻¹ of body weight and adjusted according to the plasma glucose level, which was measured every 5–10 min by a glucose analyser (2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). During the 120 min baseline period of the clamp study, glucose was maintained at fasting levels. During the 90 min target period, insulin infusion was increased to 2 milliunits·min⁻¹·kg⁻¹ of body weight. Glucose infusion rate was adjusted on the first day to maintain a normoglycaemic level (normoglycaemic target period) and, on the other experimental day (counterbalanced design), it was rapidly decreased to a glucose target of 2.7 mmol/l (hypoglycaemic target period; the levels achieved are presented in Table 1). Subjects were not informed of the glucose target level (single-blinded design).

Plasma adrenaline and noradrenaline (determined by enzyme immunoassay; IBL-Hamburg, Hamburg, Germany), insulin (determined by immunoetric assay; DPC, Los Angeles, CA, U.S.A.) and glucagon concentrations (determined by RIA; Diagnostic Products, Los Angeles, CA, U.S.A.) were assessed during baseline (90 min) and after achieving glucose target levels (75 min). Cardiovascular beat-to-beat data were assessed at baseline (80 min) and 65 min after achieving target levels. A standard three-lead ECG, intermittent blood pressure (determined by Dinamap; Criticon, Tampa, FL, U.S.A.), impedance cardiogram (determined by a modified Minnesota device; Diefenbach, Frankfurt, Germany) and respiratory frequency (determined by a respiration belt; Medizintechnik KSB, Basel, Switzerland) were recorded. Analogue-to-digital conversion was performed at 1000 Hz for off-line analysis of IBI and beat-to-beat systolic time intervals from the impedance cardiogram output (dz/dt signal; where z is the thoracic impedance). The B-point (upward deflection, representing the beginning of ventricular ejection) and X-point (minimum, corresponding to the second heart sound and closure of aortic valve) of the inverted dz/dt signal of each single beat were identified by manual control assisted by customized PC algorithms [18]. PEP was calculated from the ECG Q-onset to B-point. LVET (left ventricular ejection time) was calculated from B-point to X-point. PEP and LVET were averaged per person and over a 5 min period.

A customized computer program written in MATLAB [19] was used for computation of HF HRV (an index of cardiac vagal control). The beat-to-beat values of
Table 1 Physiological variables during baseline and the target periods
Values are means (S.E.M.). BL1, baseline on the day of normoglycaemia; T-N, target period on the day of normoglycaemia, BL2, baseline on the day of hypoglycaemia; T-H, target period on the day of hypoglycaemia. SBP, systolic blood pressure; DBP, diastolic blood pressure; RF, respiratory frequency. F (degrees of freedom) and P values are shown.

<table>
<thead>
<tr>
<th></th>
<th>BL1</th>
<th>T-N</th>
<th>BL2</th>
<th>T-H</th>
<th>F(1,14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.7 (0.1)</td>
<td>4.8 (0.1)</td>
<td>4.7 (0.1)</td>
<td>2.7 (0.1)</td>
<td>160</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean HR (beat/min)</td>
<td>69.4 (1.8)</td>
<td>71.7 (1.9)</td>
<td>71.4 (1.7)</td>
<td>71.6 (3.1)</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Mean IBI (ms)</td>
<td>875 (22)</td>
<td>847 (22)</td>
<td>849 (19)</td>
<td>863 (36)</td>
<td>2.3</td>
<td>0.15</td>
</tr>
<tr>
<td>S.D. IBI (ms)</td>
<td>57 (5.4)</td>
<td>57 (6.2)</td>
<td>52 (4.2)</td>
<td>70 (8.6)</td>
<td>8.2</td>
<td>0.01</td>
</tr>
<tr>
<td>PEP (ms)</td>
<td>104 (4)</td>
<td>92 (3)</td>
<td>101 (3)</td>
<td>54 (4)</td>
<td>78.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>LVET (ms)</td>
<td>271 (24)</td>
<td>279 (18)</td>
<td>273 (17)</td>
<td>281 (28)</td>
<td>0.0</td>
<td>0.99</td>
</tr>
<tr>
<td>HF-HRV (ln ms²)</td>
<td>6.2 (0.1)</td>
<td>6.2 (0.2)</td>
<td>6.1 (0.2)</td>
<td>6.6 (0.2)</td>
<td>6.35</td>
<td>0.02</td>
</tr>
<tr>
<td>RMSSD IBI (ms)</td>
<td>39 (4.6)</td>
<td>40 (5.6)</td>
<td>36 (4.2)</td>
<td>47 (5.6)</td>
<td>4.6</td>
<td>0.05</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120 (4)</td>
<td>121 (4)</td>
<td>125 (5)</td>
<td>124 (5)</td>
<td>0.7</td>
<td>0.42</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>66 (2)</td>
<td>66 (2)</td>
<td>69 (3)</td>
<td>58 (4)</td>
<td>19.7</td>
<td>0.0006</td>
</tr>
<tr>
<td>RF (Hz)</td>
<td>0.27 (0.02)</td>
<td>0.28 (0.01)</td>
<td>0.28 (0.01)</td>
<td>0.31 (0.02)</td>
<td>2.7</td>
<td>0.13</td>
</tr>
<tr>
<td>Adrenaline (pmol/ml)</td>
<td>0.44 (008)</td>
<td>0.45 (0.15)</td>
<td>0.49 (0.14)</td>
<td>2.84 (0.39)</td>
<td>52.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Noradrenaline (nmol/ml)</td>
<td>2.9 (0.25)</td>
<td>2.68 (0.34)</td>
<td>2.5 (0.23)</td>
<td>4.69 (0.86)</td>
<td>6.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>73 (3)</td>
<td>60 (3)</td>
<td>65 (4)</td>
<td>110 (9)</td>
<td>58.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>363 (20)</td>
<td>809 (41)</td>
<td>335 (20)</td>
<td>782 (45)</td>
<td>0.0</td>
<td>0.95</td>
</tr>
</tbody>
</table>

IBI were edited for outliers due to artefacts or ectopic myocardial activity, linearly interpolated and converted into instantaneous time series with a resolution of 4 Hz. IBI series were linearly detrended, and the power spectral densities derived for each experimental period using the Welch algorithm [20], which assembles averages of successive periodograms (a total of nine 240 point segments, overlapping by 50 %, were analysed using a Hanning window, zero-padded to length 256, and subjected to fast Fourier transformation; estimates of power were adjusted to account for attenuation produced by the Hanning window). HF HRV was computed by summing power spectral density values in the 0.15–0.45 Hz frequency range, and resulting values were normalized using the natural logarithm.

During the 5 min assessment of cardiovascular state, subjects were seated in a semi-recumbent position, instructed to close their eyes, relax and to neither move nor speak. SAS software (release 8.0; SAS, Cary, NC, U.S.A.) was used. Repeated measures ANOVA tested hypoglycaemia effects (interaction term). Non-adjusted two-tailed P values are shown. Values are means ± S.E.M.

RESULTS

The details of key variables during baseline and the target periods as shown in Table 1. Hypoglycaemia did not alter HR or LVET, but HRV increased (spectral estimate of HF HRV, as well as S.D. and RMSSD of IBI), PEP shortened (Figure 1), diastolic blood pressure decreased and adrenaline, noradrenaline and glucagon increased. Mean respiratory frequency was not significantly greater during hypoglycaemia. As expected, the doubling of the insulin infusion rate from baseline to the target period increased plasma insulin concentrations approx. 2-fold.

Figure 1 Signal averaged impedance cardiogram (−dz/dt output) referenced to the R-wave maximum during baseline and the target period (60 min)
Hypoglycaemia, but not normoglycaemia, produces shorter PEP and greater −dz/dt output maximum. The signal-averaged ECG is provided. BL1, baseline on the day of normoglycaemia; BL2, baseline on the day of hypoglycaemia; T-N, target period on the day of normoglycaemia; T-H, target period on the day of hypoglycaemia.
Plasma insulin levels were equal during hypoglycaemia and the target period on the day of normoglycaemia.

**DISCUSSION**

In the present study, prolonged hyperinsulinaemic hypoglycaemia and normoglycaemia control clamp studies were performed on two separate days according to a randomized single-blinded cross-over design. Cardiovascular variables and hormones were measured after 1 h. Increased plasma glucagon, adrenaline and noradrenaline indicated effective initiation of the counter-regulatory responses during hypoglycaemia. Shortened cardiac PEP suggested sympathetic cardiac activation during hypoglycaemia. If such a sympathetic cardiac response was induced by adrenaline infusion, it would lead to increased HR [18]. However, HR remained largely unchanged, as in other hypoglycaemic clamp studies, i.e. [7,8]. Such a response was to be expected if efferent parasympathetic cardiac nerve activity prevented tachycardia during hypoglycaemia. Indeed, increased HF HRV suggests that such a vagal mechanism helped to stabilize HR.

HR non-linearly modulates autonomic indexes [21], potentially complicating the interpretation of HRV when HR changes significantly. However, in the present study of healthy volunteers, we demonstrate that HR did not change during hypoglycaemia and, thus, this is not an issue. Respiratory frequency is a potential confounder of HRV [22], and respiratory frequency increased marginally during hypoglycaemia. If anything, this response might result in diminished HRV [22,23]; however, we found the opposite pattern, which argues against the increase in HRV during hypoglycaemia being attributable to changes in respiratory frequency.

PEP derived from non-invasive impedance cardiography has been used as an index of cardiac sympathetic nervous system activation [18,24] in previous autonomic research. Although PEP is affected by pre- and afterload changes, it is unlikely that the change in diastolic blood pressure found in the present study, as well as other hypoglycaemia studies (i.e. [9]), sufficiently explains the 45 % shortening of PEP. A decrease in blood pressure greater than those obtained in the present study was related to a < 20 % shortening of PEP [18]. Furthermore, systematic afterload changes induced by noradrenaline or tyramine infusions were found to have little impact on PEP when β-sympathetic influences were blocked pharmacologically [25]. Thus β-sympathetic stimulation seems to be far more important for the effects of PEP than blood pressure changes. Radionuclide ventriculography has revealed stable end-diastolic volumes during hypoglycaemia [9]. Thus preload changes do not play a role in hypoglycaemia effects on the cardiovascular system. The impact of glucagon on PEP seems to be of limited importance [26]; however, its impact merits further investigation.

Insulin itself impacts on neuroendocrine responses to hypoglycaemia [27], can activate the sympathetic nervous system [16] and decrease vagal influence on the heart [17]. Thus this hormone could potentially confound the results of our present study. However, plasma levels of insulin were the same during hypoglycaemia and during the appropriate control condition. Thus our findings cannot be explained by different insulin levels.

Normally functioning heart cells prefer fatty acids as fuel. In normal heart cells, carbohydrate depletion is insufficient to disturb heart cell metabolism severely enough to deteriorate pacemaker function [28], but this is not absolutely certain. However, current knowledge suggests it is unlikely that, during mild-to-moderate hypoglycaemia, HR is substantially influenced by factors other than autonomic efferents or humoral influences.

Our present findings differ from the recent study by Laitinen and co-workers [8], which suggested that cardiac parasympathetic regulation of HR is not affected by hypoglycaemia. Several methodological differences may explain this difference. The study by Laitinen et al. [8] used a paced breathing protocol at 0.2 Hz. In general, this method has some advantages, because respiratory frequency clearly has an impact on HRV [22,29] and, thus, may confound findings. However, under conditions of impaired cognitive function (such as during hypoglycaemia), it might be quite difficult to follow the external pacing protocol and, therefore, this procedure could very well be more stressful, resulting in ‘artificially’ decreased HRV [30]. More importantly, Laitinen et al. [8] used a single day fixed sequence study design (normoglycaemia, followed by hypoglycaemia). Thus there was no appropriate period controlling for influences of the time of day and duration of hyperinsulinemia. In our experience, HRV tends to decrease during longer experimental protocols. In the case of the study by Laitinen et al. [8], this burden would result in the underestimation of HRV during hypoglycaemia. This is not the case in our present study that involved a 2 day single-blinded cross-over design in which the appropriate control period was matched for time of day, duration of hyperinsulinemia and other factors. Another potentially relevant issue is that Laitinen et al. [8] targeted higher plasma glucose values (3.0 compared with 2.7 mmol/l in our present study; values are means ± S.E.M.) and, consequently, achieved lower plasma adrenaline concentrations (1.68 ± 0.32 compared with 2.84 ± 0.39 pmol/l). Finally, plasma insulin levels during normoglycaemia were higher compared with their hypoglycaemia period (999 ± 57 compared with 862 ± 33 pmol/l; values are means ± S.E.M.), thus confounding autonomic nervous system responses.

There are reports indicating that HR increases during hypoglycaemia which seem to contradict our findings.
Bolus administration of insulin tended to be associated with pronounced HR increases [9,31] and might have been due to more severe peak hypoglycaemia. In the two studies cited above, hypoglycaemia remained substantial (blood glucose < 2.5 mmol/l) 30 min after bolus insulin application and, similarly, adrenaline was significantly increased, as was cardiac sympathetic activation (increased stroke volume and left ventricular ejection fraction). Despite this cardiac sympathetic activation, HR had already returned to baseline levels. Importantly, this was not the case when the parasympathetic responses were blocked with the muscarinic inhibitor atropine. HR did not return to baseline in subjects pretreated in this way during normo- and hypoglycaemia [31]. Instead, HR remained elevated relative to normoglycaemia, suggesting that a functioning parasympathetic nervous system is required to blunt the initial hypoglycaemia-induced HR increase during prolonged hypoglycaemia.

Hypoglycaemia-induced parasympathetic activation has been shown previously in the parotid glands [32], gastrointestinal tract [33,34] and pancreas [35]; however, the present report is the first controlled study demonstrating increased parasympathetic restraint at the cardiovascular level during hypoglycaemia.

Limitations
The present study does not elucidate whether the increased vagal cardiac drive results from a change in the baroreflex or other influences. Baroreflex mechanisms might be sensitive to increased stroke volume and increased pulse pressure even when mean blood pressure is decreased.

The study design also does not allow the determination of whether the sympathetic cardiac activation originates from neural sympathetic discharge at the pacemaker cell (clearly implying dual autonomic co-activation) or from systemic adrenaline. Neural cardiac parasympathetic and sympathetic co-activation has been reported much less frequently than the dominance of one system. Previous examples of mild co-activation include responses to an aversive tone in the rat [36], in response to human touch [37] and water ingestion [13]. Such results are consistent with previous findings [38] and support the use of autonomic models allowing for co-activation and co-inhibition [24]. Whether the results of the present study provide an example of much more substantial co-activation might be clarified by more invasive research methods.

Clinical implications
There is experimental and epidemiological evidence linking sympathetic cardiac activation during severe hypoglycaemia to increased cardiac vulnerability of life-threatening arrhythmias [39] and subsequent mortality [40]. Compensatory or balancing parasympathetic effects might be quite beneficial [41]. However, parasympathetic compensation requires an intact parasympathetic neural transmission system that is not present in DAN (diabetic autonomic neuropathy). Thus it is possible that a proportion of the increased cardiovascular mortality in DAN might be explained by insufficient parasympathetic tone, leaving the heart subject to unmitigated sympathetic activation during hypoglycaemia. This possibility merits further investigation.

ACKNOWLEDGMENTS
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REFERENCES
3 Paramore, D., Fanelli, C., Shah, S. and Cryer, P. (1999) Hypoglycaemia per se stimulates sympathetic neural as well as adrenomedullary activity, but, unlike the adrenomedullary response, the forearm sympathetic neural response is not reduced after recent hypoglycaemia. Diabetes 48, 1429–1436


