Baroreflex Sensitivity Impairment During Hypoglycemia – Implications for Cardiovascular Control

Short Running Title: Baroreflex Sensitivity and Hypoglycemia

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ABSTRACT

Studies have shown associations between exposure to hypoglycemia and increased mortality, raising the possibility that hypoglycemia has adverse cardiovascular effects. In this study, we determined the acute effects of hypoglycemia on cardiovascular autonomic control. Seventeen healthy volunteers were exposed to experimental hypoglycemia (2.8 mmol/l) for 120 minutes. Cardiac vagal baroreflex function was assessed using the modified Oxford method prior to the initiation of the hypoglycemic hyperinsulinemic clamp protocol and during the last 30 minutes of hypoglycemia. During hypoglycemia compared to baseline euglycemic conditions: 1) baroreflex sensitivity decreases significantly (19.2 ± 7.5 vs. 32.9 ± 16.6 msec/mmHg, p < 0.005); 2) the systolic blood pressure threshold for baroreflex activation, increases significantly (the baroreflex function shifts to the right; 120 ± 14 vs. 112 ± 12 mmHg, p < 0.005) and 3) the maximum RR interval response (1088 ± 132 vs. 1496 ± 194 ms, p < 0.001) and maximal range of the RR interval response (414 ± 128 vs. 817 ± 183 ms, p < 0.001) decrease significantly. These findings indicate reduced vagal control and impaired cardiovascular homeostasis during hypoglycemia.
Rigorous glycemic control is the cornerstone of diabetes management, however, this results in increased episodes of iatrogenic hypoglycemia (1). Multiple community- and hospital-based studies have shown an association between exposure to hypoglycemia and increased mortality (2; 3). Furthermore, results of several large-scale clinical trials investigating the outcome of intensive glycemic control have shown either increased cardiovascular mortality or a lack of cardiovascular benefit (4-7). These results raise the possibility that exposure to hypoglycemia has adverse and unknown effects that persist after hypoglycemia resolves and may oppose cardiovascular benefits of improved glycemic control (8; 9).

Prior exposure to hypoglycemia impairs the hormonal and muscle sympathetic nerve activity (MSNA) response to subsequent hypoglycemia (10), the hormonal responses to subsequent exercise (11), and autonomic control of cardiovascular function (12). After exposure to hypoglycemia, induced by the hypoglycemic hyperinsulinemic clamp protocol, individuals exhibit decreased baroreflex sensitivity (BRS), decreased MSNA response to transient hypotension, and decreased norepinephrine response to orthostatic stress as compared to prior exposure to euglycemic hyperinsulinemic conditions. These changes in autonomic cardiovascular control are present in the euglycemic state, 16 hours after hypoglycemic exposure (12). The time of onset for these changes is not established.

The baroreflex plays a central role in maintaining cardiovascular homeostasis. Impaired baroreflex function is associated with increased mortality in patients with diabetes, hypertension, and cardiovascular disease (13-15). Thus, hypoglycemia-induced changes in autonomic cardiovascular control could contribute to the mortality associated with hypoglycemia (7; 8; 16).
The modified Oxford test is the gold standard for characterizing the baroreflex. By assessing baroreflex function across a wide range of clinically relevant blood pressures, this test defines the full hemodynamic range of baroreflex engagement. The modified Oxford method has not been used to assess BRS during hypoglycemia.

The purpose of this study was to test the hypothesis that impairment in baroreflex cardiovascular control begins during hypoglycemia. We therefore assessed baroreflex function using the modified Oxford method before and during a hypoglycemic hyperinsulinemic clamp protocol in healthy participants.

**RESEARCH DESIGN AND METHODS**

**Study population.**

Healthy men and women, aged 18 to 40 years, were recruited from the greater Boston area. Exclusion criteria included: evidence of any medical illness on history or physical examination; BMI > 30 kg/m2; tobacco use; substance abuse; pregnancy; lactation; menopause; abnormalities on electrocardiogram; or abnormal blood electrolyte, liver function test, complete blood count or urinalysis values. The study protocol (NCT01394627) was approved by the Partners Institutional Review Board and all subjects provided written, informed consent.

**Diet and activity.**

Participants stopped over-the-counter medications two weeks before admission and refrained from vigorous exercise from one week before admission to the Center for Clinical Investigation
at Brigham and Women’s Hospital. Participants consumed an isocaloric diet (125 mmol/day Na+, 125 mmol/day K+, 200 mmol/day Ca++) lacking foods high in monoamines beginning four days prior to and continuing throughout the admission. A hypoglycemic hyperinsulinemic clamp procedure was performed in the morning with participants having been supine and fasting since midnight.

**Hypoglycemic hyperinsulinemic clamp protocol**

As previously described, participants received a primed continuous intravenous infusion of regular insulin at 80 mU/m2 body surface area/min for ~150 minutes (all participants received Humulin R [Eli Lilly, Indianapolis, IN, USA] except one who received Novolin R [NovoNordisk, Princeton, NJ, USA]). Twenty percent dextrose was infused intravenously to achieve blood glucose of 2.8 mmol/L (50 mg/dL) for 120 minutes (17). Blood samples were withdrawn through an indwelling intravenous catheter placed in a retrograde fashion in the participant’s wrist/hand that rested in a warm box (150°F) throughout the procedure. Plasma glucose was assessed every 5 min using a bedside glucose analyzer (YSI 2300 Stat Plus Glucose and Lactate Analyzer, YSI, Yellow Springs, OH, USA). Blood was collected for analysis of insulin at baseline and during the insulin infusion.

**Baroreflex assessment**

Cardiac vagal baroreflex function was assessed using the modified Oxford method (18) at baseline and during the last 30 minutes of the hypoglycemic clamp (See Figure 1). Following a baseline measurement of blood pressure and heart rate utilizing an automated oscillometric blood pressure monitor (Dinamap, Critikon Company, Tampa, FL), participants received intravenous
bolus injections of 100 micrograms sodium nitroprusside followed 60 seconds later by 150 micrograms phenylephrine hydrochloride as previously described (12). This procedure causes a decrease in systolic blood pressure (SBP) of ~15 mmHg below baseline SBP followed by an increase of ~15 mmHg above baseline. RR interval and beat-to-beat blood pressure were measured using the Finometer (FMS, Amsterdam, the Netherlands). Baroreflex assessments were performed in duplicate. The minimum RR interval and maximum RR interval were used to define the range of heart rate response during the baroreflex function assessment. Baroreflex sensitivity (BRS) was determined by the slope of the relation between RR interval and SBP. The threshold of baroreflex function was defined as the SBP at which the RR interval started to increase after reaching its minimum value during the modified Oxford test. The saturation point of baroreflex function was defined as the SBP at which the RR interval reached its maximum value (See Figs 2 and 3).

Statistical analysis.

Data were analyzed using Student’s two-tailed t test and ANOVA with repeated measures (General Linear model, SPSS). Categorical variables were compared using Fisher’s exact test. Data are expressed as mean ± standard deviation unless specified otherwise. A p-value less than 0.05 was considered statistically significant. All statistics were performed with SAS 9.3 (SAS Institute, Cary, NC), JMP Pro 10.0 (SAS Institute, Cary, NC) or SPSS.

RESULTS

Subject demographics and clamp data
Seventeen participants (age 26 ± 6 years, 76% male, 59% Caucasian, BMI 23.2 ± 3.6 kg/m2) were included in the analysis. Four additional participants were excluded due to incomplete autonomic testing data (n=3) or average blood glucose > 3.0 mmol/l during the final 120 min of the clamp protocol (n=1).

All 17 participants were insulin sensitive (average homeostatic model assessment of 1.05 ± 0.42 and fasting glucose of 5.0 ± 0.3 mmol/L). Insulin infusion increased serum insulin from baseline levels of 29 ± 11 pmol/l to 731 ± 290 pmol/l. Figure 1 shows plasma glucose levels during the clamp procedure and timing of the modified Oxford test. The average plasma glucose during the 120 min of hypoglycemia was 2.8 ± 0.1 mmol/l.

**Baroreflex assessment before and during hypoglycemia**

Blood pressure and heart rate were assessed prior to the modified Oxford procedures at baseline, when subjects were euglycemic, and during the hypoglycemic clamp (See Figure 1). Systolic blood pressures were similar, whereas diastolic blood pressure was significantly lower, under hypoglycemic compared to baseline euglycemic conditions (SBP \(_{\text{baseline}}\) 116 ± 11 mmHg vs. SBP \(_{\text{hypoglycemia}}\) 119 ± 14 mmHg, p=NS; DBP \(_{\text{baseline}}\) 67 ± 7 mmHg vs. and DBP \(_{\text{hypoglycemia}}\) 61 ± 7 mmHg, p<0.001). Heart rate (HR) increased significantly during hypoglycemia compared to baseline euglycemia (HR \(_{\text{baseline}}\) 60± 8 beats per minute vs. 71 ± 8 beats per minute; p<0.001).

The minimum and maximum (peak and trough) systolic blood pressures during the modified Oxford procedure were similar under hypoglycemic and baseline euglycemic conditions (See Table 1). Analysis of baroreflex function revealed significant differences between baseline and
hypoglycemic conditions (see Figure 2 for representative subject, Figure 3 and Table 1 for group data). Baroreflex sensitivity decreased during hypoglycemia compared to baseline as shown by the decrease in the slope of the linear portion of the sigmoidal baroreflex function plot (19.2 ± 7.5 msec/mmHg during hypoglycemia vs. 32.9 ± 16.6 msec/mmHg at baseline, p < 0.005).

The systolic blood pressure threshold for activation of the baroreflex increased significantly (a shift to the right of the baroreflex plot) during hypoglycemia compared to baseline euglycemia, (see Figure 2, Figure 3 and Table 1); indicating higher blood pressures were required for baroreflex activation. The saturation point of the baroreflex curve (the point at which blood pressure increases elicited no further increases in RR interval) was similar during baseline euglycemia compared to hypoglycemia (see Figure 2, Figure 3 and Table 1).

The maximum RR interval response elicited by the increase in blood pressure during the modified Oxford procedure decreased significantly (a downward shift of the baroreflex plot) during hypoglycemia compared to baseline (see Figure 2, Figure 3 and Table 1). Further, the range between the maximum RR interval and minimum RR interval was significantly decreased during hypoglycemia versus baseline, indicating a blunted heart rate response to pharmacological induced changes in blood pressure (see Figure 2, Figure 3 and Table 1).

**DISCUSSION**

Our data demonstrate that during insulin-induced hypoglycemia (1) BRS is decreased; (2) the blood pressure threshold for baroreflex activation is increased; and (3) the maximum RR interval response and maximal range of the RR interval responses are decreased. These findings indicate an inability to maintain optimal vagal control during hypoglycemia. Impaired cardiovascular
homeostasis during hypoglycemia could contribute to the adverse cardiac outcomes associated with hypoglycemia. These results extend our previous findings, which showed a decrease in BRS sixteen hours after exposure to hypoglycemia (12). Taken together these two studies suggest that hypoglycemia-mediated attenuation of cardiac vagal baroreflex function begins during hypoglycemia and persists for at least 16 hours after hypoglycemia has ended.

Patients with diabetes are often exposed to asymptomatic episodes of hypoglycemia. The incidence of severe hypoglycemic episodes (those that require medical assistance) may be as high as 3.2 episodes per patient per year in individuals with type 1 diabetes, and 0.1 to 0.7 episodes per patient per year in individuals with type 2 diabetes (19; 20). These episodes can last up to 10% of a 24-hour period (21). There is an increased interest in the potential adverse effects of hypoglycemia prompted by studies showing associations between severe hypoglycemia and cardiovascular disease and microvascular disease in individuals with type 2 diabetes (9), and by large-scale clinical studies showing an association between intensive glycemic control and adverse cardiovascular events (4-6). During hypoglycemia, multiple processes associated with cardiovascular injury or dysfunction are induced. These include increased activation of the renin-angiotensin-aldosterone system (22); increases in inflammatory cytokines including IL-6, IL-8, TNF alpha, and endothelin-1 (17; 23; 24); endothelial dysfunction (25); QT interval prolongation (26); cardiac arrhythmias (16); decrease in the spontaneous baroreflex (27); and increased sympathetic nerve activity (28). All these factors could have a role in the adverse clinical outcomes associated with hypoglycemia.
The present finding that autonomic control of cardiovascular function is altered during hypoglycemia extends our understanding of the potential adverse cardiovascular effects of hypoglycemia. We used pharmacological provocations to fully characterize the baroreflex across a range of physiologically relevant blood pressures, allowing us to determine the specific changes in baroreflex control induced by hypoglycemia. Our observation that BRS is reduced during hypoglycemia over a wide range of blood pressures extends and supports a prior study of spontaneous cardiac baroreceptor sensitivity during hypoglycemia (27). In addition, we show directly, for the first time, that during hypoglycemia, both the increase in the blood pressure threshold for activation of the baroreflex and the decrease in the range of heart rate responses to blood pressure changes contribute to the altered autonomic heart rate control during hypoglycemia. These mechanisms indicate an inability to maintain optimal vagal control leading to a higher probability of impaired cardiovascular homeostasis and adverse cardiac events during hypoglycemia. The observation that alteration of baroreflex function is present within 90-120 minutes of exposure to hypoglycemia and with exposure to a relatively moderate degree of hypoglycemia (2.8 mmol/l) increases the relevancy to clinical care.

The baroreflex plays a pivotal role in cardiovascular homeostasis. Decreases in BRS lead to an impaired homeostatic response to hemodynamic stress. Baroreflex dysfunction is also associated with an increased risk of cardiac arrhythmias (29) and is a predictor of mortality in the post-myocardial infarction period (15) and in individuals with type 2 diabetes (13). Thus, hypoglycemia-induced changes in baroreflex function could have significant clinical implications that may be particularly relevant in individuals with diabetes who experience hypoglycemia or have underlying autonomic dysfunction. While the current studies were
performed in healthy subjects our results are consistent with published studies in individuals with diabetes. In individuals with type 1 diabetes, acute hypoglycemia reduces measures of autonomic and cardiovascular function (30). Further, indices of hypoglycemia derived from continuous glucose monitoring were associated with reduced heart rate variability in individuals with type 1 diabetes (31).

Several studies suggest that activation of carotid body chemoreceptors by hypoxia reduces baroreflex sensitivity (32) or shifts the baroreflex stimulus response curves to higher blood pressures and heart rates (33; 34). When studied in individuals with type 1 diabetes, acute hypoxia further deteriorates hypoglycemia-evoked decreases in the spontaneous cardiac baroreflex and measures of heart rate variability (35). This may be relevant in patients with sleep apnea, a common condition in individuals with diabetes (36).

There are some limitations to our study. We cannot identify the exact mechanisms that underlie the decreased baroreflex sensitivity, increased blood pressure threshold and decreased maximum RR interval response. This requires further studies. However, the changes in the baroreflex are not due to differences in the blood pressure provocation since the blood pressure range and blood pressure saturation point were similar during baseline euglycemia and hypoglycemia. Additionally, the current study cannot determine the relative roles played by hyperinsulinemia and hypoglycemia in the observed impairments in the cardiovagal baroreflex. Insulin, in the presence of euglycemia, induces vasodilation and increases sympathetic activity (37), and, when administered centrally to anesthetized rats, increases baroreflex gain of heart rate and MSNA (38; 39). In contrast, in a study in humans, hyperinsulinemia, in the presence of euglycemia,
increased the MSNA baroreflex gain (i.e., the MSNA response to spontaneous changes in blood pressure) but did not modify cardiovagal baroreflex gain (i.e., the heart rate response to spontaneous changes in blood pressure) (40). These findings contrast with our observation that insulin-induced hypoglycemia blunts the cardiovagal baroreflex, and support the hypothesis that blunting of the cardiovagal baroreflex is due to hypoglycemia, not hyperinsulinemia. Further studies are necessary to prove this point.

In summary, these data suggest that during acute insulin-induced hypoglycemia cardiac vagal baroreflex is impaired in healthy non-obese individuals. Further studies are needed to determine whether these changes occur in individuals with diabetes (41) and to determine the relevance of hypoglycemia-induced alterations in autonomic control of cardiovascular function to clinical outcomes.
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Duality of Interest

No potential conflicts of interest relevant to this article were reported.

Author Contributions

ADR conceived the idea, recruited participants, conducted the study, interpreted data, and wrote the manuscript. IB interpreted data and wrote the manuscript. JD, MBG, and LK helped in recruiting participants and conducted the study. SB helped in conducting the study. RF and GKA conceived the idea, interpreted data, and wrote the manuscript. All authors contributed to the manuscript and take full responsibility for its originality. ADR, RF and GKA are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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Table 1. Characteristic points of modified Oxford baroreflex function for all subjects during baseline euglycemia and hypoglycemia

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Hypoglycemia</th>
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<tr>
<td>Blood Pressure (mm Hg)</td>
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<td>SBP minimum</td>
<td>98 ± 13</td>
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<td>SBP threshold</td>
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<td>SBP maximum</td>
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<td>RR maximum</td>
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<td>RR range</td>
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<td>414 ± 128**</td>
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<td>BRS (ms/mm Hg)</td>
<td>32.9 ± 16.6</td>
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* Hypoglycemia vs. Baseline, p<0.005

** Hypoglycemia vs. Baseline, p<0.001
FIGURE LEGENDS

Figure 1. Timing of the baroreflex assessment (Modified Oxford) relative to the plasma glucose levels (mean ± SD) during the hypoglycemic hyperinsulinemic clamp protocol. Straight line represents the target glucose level (2.8 mmol/L).

Figure 2. Baroreflex function from a representative subject measured by the modified Oxford method during baseline euglycemia (closed circles) and hypoglycemia (open triangles). Data points represent the corresponding values of systolic blood pressure (SBP) and cardiac interval (RR) during the pharmacologically-induced blood pressure change. For baroreflex assessment, RR is plotted as a function of SBP between the lowest and the highest SBP values. The linear portion (arrows) of the sigmoid function describes baroreflex sensitivity (see Methods). During hypoglycemia baroreflex sensitivity is decreased, maximal RR interval is decreased and the sigmoidal baroreflex function curve shifts to the right.

Figure 3: Baroreflex function measured by the modified Oxford method during baseline euglycemia (closed circles) and hypoglycemia (open triangles). Group average values for the threshold and saturation blood pressures and corresponding cardiac intervals (RR) are displayed for each glycemic condition. A sigmoidal curve is fitted to these group data for both glycemic conditions. Horizontal error bars denote standard deviation of blood pressure, and vertical error bars denote standard deviation of the RR interval.

The baroreflex threshold shifts to higher blood pressures during hypoglycemia (arrow 1); the RR interval at the blood pressure saturation point decreases during hypoglycemia (arrow 2); and the RR interval range is lower during hypoglycemia (double-headed arrows 3). Data for baroreflex sensitivity (the slope of the linear portion of the baroreflex sigmoid curve) showing a significant decrease with hypoglycemia is displayed in Table 1.

* denotes the difference in the baroreflex blood pressure threshold, hypoglycemia vs. baseline, P<0.005
† denotes difference in maximum RR interval at the blood pressure saturation point, hypoglycemia vs. baseline, P<0.001
** denotes difference in RR interval ranges, hypoglycemia vs. baseline, P<0.001
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