Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects

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Van de Borne, Philippe, Martin Hausberg, Robert P. Hoffman, Allyn L. Mark, and Erling A. Anderson. Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R178–R183, 1999.—The exact mechanisms for the decrease in R-R interval (RRI) during acute physiological hyperinsulinemia with euglycemia are unknown. Power spectral analysis of RRI and microneurographic recordings of muscle sympathetic nerve activity (MSNA) in 16 normal subjects provided markers of autonomic control during 90-min hyperinsulinemic/euglycemic clamps. By infusing propranolol and insulin (n = 6 subjects), we also explored the contribution of heightened cardiac sympathetic activity to the insulin-induced decrease in RRI. Slight decreases in RRI (P < 0.001) induced by sevenfold increases in plasma insulin could not be suppressed by propranolol. Insulin increased MSNA by more than twofold (P < 0.001), decreased the high-frequency variability of RRI (P < 0.01), but did not affect the absolute low-frequency variability of RRI. These results suggest that reductions in cardiac vagal tone and modulation contribute at least in part to the reduction in RRI during hyperinsulinemia. Moreover, more than twofold increases in MSNA occurring concurrently with a slight and not purely sympathetically mediated tachycardia suggest regionally nonuniform increases in sympathetic activity during hyperinsulinemia in humans.

Studied in normal humans have shown that acute physiological increases in plasma insulin in the absence of changes in blood glucose cause opposing sympathexcitation and vasodilatation in the skeletal muscle vascular bed (2, 3). Furthermore, hyperinsulinemia increases heart rate and cardiac output, which may be secondary to the peripheral vasodilator actions of insulin (2, 3, 6, 28). The insulin-induced increase in heart rate may be caused by increased cardiac sympathetic outflow but also by cardiac vagal withdrawal. A previous study in animals (9) suggested that chronic hyperinsulinemia is accompanied by reduced cardiac vagal activity. The exact mechanisms of insulin-induced increases in heart rate have thus far not been assessed in humans. Spectral analysis of R-R interval (1, 30) and β-adrenergic receptor blockade during a hyperinsulinemic/euglycemic clamp could be used to address this issue.

R-R interval discloses cyclic variations related to the respiratory cycle called respiratory sinus arrhythmia or high-frequency (HF) variability (1, 30). In addition, R-R interval discloses also low-frequency (LF) oscillations, and changes in the amplitude of these cyclic variations provide markers of cardiac vagal and sympathetic modulation (1, 10, 23, 25, 30). In the present study, we performed a hyperinsulinemic/euglycemic clamp in normal human volunteers and measured heart rate, blood pressure, and respiration. R-R interval variability was determined by power spectral analysis.

The insulin-induced changes in HF variability of R-R interval were assessed to test the hypothesis that hyperinsulinemia decreases cardiac vagal modulation.

We also administered a continuous intravenous infusion of propranolol, a β-adrenergic receptor blocker, during the hyperinsulinemic/euglycemic clamp to determine if increased cardiac sympathetic outflow contributed to the increase in heart rate induced by insulin.

In addition, animal studies have suggested that insulin may produce nonuniform increases in sympathetic activity (20), and changes in cardiac sympathetic activity during hyperinsulinemia were compared with those present in direct recordings of sympathetic nerve traffic to muscle circulation.

METHODS

Subjects. We studied 16 subjects with a mean age of 31 ± 1 (SE) yr (5 males, 11 females) and a mean body mass index of 24 ± 1 kg/m². All subjects were healthy and had blood pressures <130/80 mmHg measured on three different occasions in the sitting position.

None was receiving any medication. The study was approved by the Institutional Human Subjects Review Committee, and written informed consent was obtained.

Measurements. Blood pressures were measured with an automatic sphygmomanometer (Lifestat 200; Physio-Control, Redmond, WA). Electrocardiogram and respiration (Biotach and Pneumotrace; Gould Electronics, Valley View, OH) were recorded on all subjects. Forearm blood flow was measured by venous occlusion plethysmography using air-filled latex cuffs. Intraneural recordings of muscle sympathetic nerve activity (MSNA) were obtained from leg microneurographic recordings of multunit postganglionic sympathetic fibers, measured in the peroneal nerve posterior to the fibular head (19, 34). We used tungsten microelectrodes (shaft diameter 200 µm, tapering to an uninsulated tip of 1–5 µm). A subcutaneous reference electrode was inserted 2–3 cm away from the recording electrode inserted in the nerve fascicle. The neural signals were amplified, filtered, rectified, and integrated to...
obtain a mean voltage display of sympathetic nerve activity. All nerve recordings met standard criteria (19, 34).

Electrocardiogram, sympathetic neurogram, and respiration were recorded on a Macintosh Quadra 900 Computer (Apple Computer, Cupertino, CA) with a MacLab 8/8 data acquisition system (AD Instruments, Milford, MA).

These measurements were also recorded on an IBM 433DX/T computer. All subjects were breathing spontaneously and were not allowed to speak during the study.

Spectral analysis. Analog-to-digital conversion was performed online at 1,000 samples/s for the electrocardiogram and at 200 samples/s for the respiratory signal. Stationary segments devoid of arrhythmias and artifacts (150–300 s) were then analyzed offline with a personal computer (IBM 433DX/T).

The principles of the software for data acquisition and spectral analysis have been described elsewhere (21, 22, 27). A discrete Fourier transformation (CARSPAN 1.0; Faculty of Psychology, University of Groningen, Groningen, the Netherlands) with a spectral resolution of 0.01 Hz was applied to the time series of R-R interval. A respirogram synchronized with the tachogram was also obtained by sampling the signal of respiratory activity once every cardiac cycle.

Absolute power spectral density functions (ms²) were computed for R-R interval in the 0.02- to 0.50-Hz band (total variability), in the very-low-frequency (VLF) band (0.02–0.03 Hz), in the LF band (0.04–14 Hz), and in the HF band (0.15–0.35 Hz).

The respirogram underwent a similar analysis to the one described above.

Hyperinsulinemic/euglycemic clamp. Insulin (Humulin; Eli Lilly, Indianapolis, IN) diluted in saline with 1 ml of the subject's blood was infused intravenously by a digital infusion pump (Bard MedSystems, North Reading, MA). A hyperinsulinemic/euglycemic clamp over 90 min was performed as previously described (2, 3). Insulin infusion rates were targeted to produce plasma insulin levels of 85 ìU/mL. Fasting plasma glucose levels were maintained by variable 20% dextrose infusion using an infusion pump (Flo-gard 6200; Travenol Laboratories, Deerfield, IL).

Plasma glucose and insulin were determined every 5 min. Plasma glucose was analyzed by a YSI glucose analyzer (model 27; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin levels were determined by radioimmunoassay (35).

Protocol. All studies were started at 7:30 AM after an overnight fast. Subjects were placed in the supine position and instrumented for measurement of heart rate, blood pressure, and respiration. Intravenous catheters were placed in the right and left antecubital fossa for insulin/glucose infusion and blood sampling, respectively. The right arm was instrumented for forearm blood flow measurement. After an acceptable sympathetic nerve recording site was achieved, 10 min of baseline recordings were obtained before performing the 90-min hyperinsulinemic/euglycemic clamp.

We repeated 90-min hyperinsulinemic/euglycemic clamps during a continuous infusion of propranolol in 6 of the 16 normal subjects. We wanted thereby to determine if increased cardiac sympathetic outflow and/or cardiac vagal withdrawal contribute to the reduction in R-R interval during hyperinsulinemia. Measurements were performed during a 10-min baseline period before an intravenous injection of 5 mg of propranolol (26). A continuous infusion of 80 ìg/min of propranolol was then started (26) and continued throughout the protocol, namely a baseline period of 20 min and a 90-min hyperinsulinemic/euglycemic clamp.

Vehicle control studies were performed in six normal subjects (5 males, mean age 28 ± 3 yr, mean body mass index 26 ± 2 kg/m²) in whom 0.2% saline was infused in a comparable volume to the insulin session.

Data analysis. Measurements were analyzed in a blinded fashion during 10 min of baseline recordings and during the last 15 min of the hyperinsulinemic/euglycemic clamp or vehicle session. The baseline plasma glucose level of 85 ± 2 mg/dl did not change significantly during insulin infusion (89 ± 2 mg/dl at 90 min, P = not significant [NS]). R-R interval decreased from 1,047 ± 49 to 949 ± 45 ms after 90 min of insulin infusion (P < 0.001; Fig. 1 and Table 1). Insulin increased forearm blood flow from 2.1 ± 0.2 to 3.4 ± 0.4 ml · min⁻¹ · 100 ml⁻¹ (P < 0.01; Fig. 1). Mean blood pressure was 85 ± 3 mmHg during baseline and 85 ± 3 mmHg after 90 min of insulin infusion. Consequently, insulin infusion decreased baseline forearm vascular resistance of 46 ± 5 units to 31 ± 4 units at the end of the clamp (P < 0.01; Fig. 1). Insulin also caused a marked increase in mean sympathetic nerve activity (MSNA) from 208 ± 36 U/min (15 ± 2 bursts/min) at baseline to 433 ± 76 U/min (25 ± 2 bursts/min) during insulin (both P < 0.001; Fig. 1).

Insulin decreased the absolute HF variability of R-R interval (P < 0.01; Fig. 2 and Table 1). This was not due to a change in the respiratory pattern during insulin, since respiratory rate was 0.29 ± 0.01 Hz during baseline and 0.28 ± 0.01 Hz during insulin (P = NS). Moreover, qualitative assessment of minute ventilation by the use of the pneumograph suggested that tidal volume increased during hyperinsulinemia as respiratory power increased from 6.5 ± 1.2 AU² during baseline to 9.6 ± 2.2 AU² during insulin infusion (P < 0.05) in the absence of changes in respiratory rate. The HF respiratory variability accounted for 84 ± 1 and 82 ± 3% of the respiratory power during baseline and insulin infusion, respectively (P = NS). Insulin did not affect the absolute VLF and LF variabilities of the R-R interval.

Propranolol. β-Blockade increased the baseline R-R interval from 1,103 ± 42 to 1,213 ± 27 ms (P < 0.05, Table 2). Mean blood pressure and forearm vascular resistance remained unchanged. Propranolol did not...
suppress the decrease in R-R interval induced by insulin. R-R interval decreased from 1,213 ± 27 ms during the infusion of propranolol to 1,146 ± 33 ms (P < 0.05) during the last 15 min of infusion of insulin and propranolol. Mean blood pressure was 81 ± 2 mmHg during the infusion of propranolol and 78 ± 2 mmHg at the end of the infusion of insulin and propranolol (P < 0.05). Forearm vascular resistance was 41 ± 5 units during the infusion of propranolol and decreased to 31 ± 3 units at the end of the infusion of insulin and propranolol (P < 0.05).

Vehicle control studies. R-R interval was 1,072 ± 65 ms during baseline and 1,077 ± 80 ms during the last 15 min of vehicle infusion (P = NS). Vehicle did not affect forearm blood flow (2.5 ± 0.4 ml·min⁻¹·100 ml⁻¹ during baseline and 2.5 ± 0.3 ml·min⁻¹·100 ml⁻¹ during vehicle, P = NS) and forearm vascular resistance (38 ± 6 units during baseline and 39 ± 5 units during vehicle, P = NS). Mean blood pressure was 85 ± 3 mmHg during baseline and was 89 ± 3 mmHg during the last 15 min of vehicle infusion (P = 0.03). However, vehicle did not affect MSNA. MSNA was 269 ± 42 U/min (20 ± 1 bursts/min) at baseline and 261 ± 61 U/min (16 ± 3 bursts/min) at the end of vehicle infusion (P = NS). R-R interval variability remained unchanged during vehicle infusion. In particular, the absolute HF variability in R-R interval was 3,742 ± 2,901 ms² during vehicle infusion.

DISCUSSION

In this study, we determined the effects of insulin on the variability of R-R interval. Our study reveals that insulin decreases cardiac vagal tone since the reduction in R-R interval induced by insulin could not be abolished by propranolol. Moreover, insulin decreased also the absolute HF variability of R-R interval but did not affect the absolute LF variability of R-R interval. The HF variability of R-R interval is a marker of cardiac vagal modulation (10, 14, 16, 17, 25) and can be affected by changes in the respiratory-related vagal modulation of R-R interval (breathing pattern; see Refs. 10, 14, 16, 17, 32). However, respiratory rate remained unchanged, and qualitative assessment of respiration suggested that tidal volume increased during hyperinsulinemia, as a possible consequence of increased oxygen consumption during sympathetic hyperactivity (33). Thus our study reveals that reductions in the HF variability in R-R interval were not due to smaller tidal

Table 1. RRI, absolute total VLF, LF, and HF variabilities of RRI, MBP, FBF, FVR, and MSNA at baseline and after 90 min of insulin infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Insulin</th>
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<tr>
<td>RRI, ms</td>
<td>1.047 ± 49</td>
<td>0.949 ± 45*</td>
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<tr>
<td>Total RRI variability, ms²</td>
<td>4.005 ± 921</td>
<td>3.033 ± 726</td>
</tr>
<tr>
<td>VLF RRI variability, ms²</td>
<td>547 ± 103</td>
<td>551 ± 65</td>
</tr>
<tr>
<td>LF RRI variability, ms²</td>
<td>1,290 ± 275</td>
<td>1,142 ± 223</td>
</tr>
<tr>
<td>HF RRI variability, ms²</td>
<td>2,110 ± 598</td>
<td>1,261 ± 494†</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>85 ± 3</td>
<td>85 ± 3</td>
</tr>
<tr>
<td>FBF, ml·min⁻¹·100 ml⁻¹</td>
<td>2.1 ± 0.2</td>
<td>3.4 ± 0.4†</td>
</tr>
<tr>
<td>FVR, units</td>
<td>46 ± 5</td>
<td>31 ± 4†</td>
</tr>
<tr>
<td>MSNA, burst/min</td>
<td>15 ± 2</td>
<td>25 ± 2*</td>
</tr>
<tr>
<td>Amplitude, units</td>
<td>208 ± 36</td>
<td>433 ± 76*</td>
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</table>

Data are means ± SE; n = 16 subjects. RRI, R-R interval; VLF, very low frequency; LF, low frequency; HF, high frequency; MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; MSNA, muscle sympathetic nerve activity. *P < 0.001 and †P < 0.01, insulin vs. baseline.

Fig. 1. Tracings of electrocardiogram (EKG), mean voltage neurogram, and forearm plethysmogram at baseline (left) and after 90 min of insulin infusion (right) in one subject. Insulin increased heart rate (HR) and induced a marked increase in muscle sympathetic nerve activity (MSNA). FBF, forearm blood flow.
volumes during hyperinsulinemia (10, 14, 16, 17, 32) and that insulin decreased not only cardiac vagal tone but also cardiac vagal modulation.

Previous studies on heart rate variability during hyperinsulinemia (7, 11) neither recorded MSNA nor administered a β-adrenergic receptor blocker during hyperinsulinemic/euglycemic clamps. In contrast, our study reveals that propranolol does not abolish the decrease in R-R interval induced by insulin and that insulin increases heart rate only by 6 beats/min at a time when sympathetic drive to muscle circulation is increased by more than twofold. Thus our results suggest that a reduction in cardiac vagal tone contributed at least in part to the small increase in heart rate during hyperinsulinemia and that insulin produces differential increases in sympathetic activity in humans. These results support previous observations in animals in which insulin increased lumbar but not renal sympathetic nerve activity (20) and in humans in which insulin increased sympathetic nerve activity to muscle but not to skin (8). This finding could also explain why heart rate remained unchanged during hyperinsulinemia in the study of Bellavere et al. (7) and increased only by two beats per minute in the study of Emdin et al. (11).

Insulin sympathoexcitatory effects are mediated at least in part by a central neural action and possibly by baroreflex mechanisms secondary to peripheral vasodilatation (28). Increased sympathetic drive during hyperinsulinemic-euglycemic clamps is primarily targeted at the skeletal muscle vasculature. In addition, there is evidence that insulin selectively stimulates blood flow and decreases vascular resistance in skeletal muscle, chiefly by potentiation of endothelium-dependent vaso-

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Table 2. Group data measurements of RRI, MBP, FBF, and FVR during baseline, propranolol infusion, and infusion of both propranolol and insulin

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Propranolol</th>
<th>Propranolol + Insulin</th>
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</thead>
<tbody>
<tr>
<td>RRI, ms</td>
<td>1,103±42</td>
<td>1,213±27*</td>
<td>1,146±33†</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>80±2</td>
<td>81±2</td>
<td>78±2†</td>
</tr>
<tr>
<td>FBF, ml·min⁻¹·100 ml⁻¹</td>
<td>2.3±0.2</td>
<td>2.2±0.3</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>FVR, units</td>
<td>39±5</td>
<td>41±5</td>
<td>31±3†</td>
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</table>

Data are means ± SE; n = 6 subjects. *P < 0.05 vs. baseline. †P < 0.05 vs. propranolol alone.
sympathetic activity in humans. Accordingly, blood pressure did not change during hyperinsulinemia in our study as a result of opposing pressor and depressor actions of insulin in the skeletal vasculature. We also reported similar small changes in blood pressure during hyperglycemic/euglycemic clamps in two previous studies (2, 3). Regionally nonuniform increases in sympathetic drive opposing the vasodilatory action of insulin (2, 3, 28, 29) could explain why more than twofold increases in sympathetic nerve activity to muscle circulation were not accompanied by the expected increases in blood pressure.

Perspectives

The continuing difficulty with research on acute effects of increases in insulin on autonomic control is to establish relevance to the chronic hyperinsulinemia of obesity (33). There are, however, several studies that also suggest that cardiac vagal outflow is decreased, in the absence of a heightened cardiac sympathetic outflow, during chronic hyperinsulinemia. First, there is recent evidence of nonuniform sympathetic activation in obese subjects in whom cardiac sympathetic activity, directly assessed by norepinephrine spillover techniques, is depressed (33). Second, sequential parasympathetic blockade with atropine and sympathetic blockade with esmolol have revealed that weight gain impairs more cardiac parasympathetic control than cardiac sympathetic control in nonobese and obese subjects (5). Third, similar direct evidence of increased heart rate as a result of cardiac parasympathetic withdrawal rather than increased sympathetic stimulation was also found in obese dogs (31). Last, two other studies in which heart rate variability provided indirect measures of cardiac autonomic control also concluded that heart rate increases as a result of parasympathetic withdrawal when body weight increases (15, 24).

Thus our data may provide a mechanistic explanation for previous observations of cardiac parasympathetic withdrawal in the absence of cardiac sympathetic activation in obese hyperinsulinemic subjects. The exact mechanisms by which obesity may have differential effects on cardiac sympathetic and vagal control are unknown. It is believed that central nervous mechanisms for sympathetic activation in obesity may reside in the hypothalamus (33), with neuropeptide Y being an important neurotransmitter (33, 13) that also possesses vagal inhibitory capacities (36). Finally, there is indirect evidence that decreased cardiac vagal activity precedes onsets of ventricular tachycardia (12), and it is therefore tempting to speculate that insulin-induced cardiac vagal withdrawal might contribute to the increased cardiovascular mortality of obesity.

In conclusion, this study suggests that acute physiological elevations in plasma insulin decrease cardiac vagal tone and modulation and that insulin produces regionally nonuniform increases in muscle and cardiac sympathetic activity in humans.

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