Occurrence of Dawn Phenomenon Without Change in Insulin Clearance in Patients With Insulin-Dependent Diabetes Mellitus

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SUMMARY

To assess the possible role of increased insulin clearance in the pathogenesis of the dawn phenomenon, we compared plasma free-insulin concentrations, free-insulin clearance rates, and plasma glucose concentrations in eight subjects with insulin-dependent diabetes mellitus (IDDM) during infusion of insulin from midnight to 0800 h (0.15 mU · kg^{-1} · min^{-1}) with a Biostator and a Harvard pump. During infusion of insulin with the Biostator, plasma free insulin decreased 40% (from 14 ± 1 to 9 ± 1 μU/ml, $P < .01$), insulin clearance increased 54% (from 11 ± 1 to 17 ± 2 ml/min, $P < .05$), and plasma glucose increased from 101 ± 4 to 217 ± 27 mg/dl, $P < .01$. During infusion of insulin with the Harvard pump, neither plasma free insulin (14 ± vs. 13 ± 1 μU/ml) nor free-insulin clearance (12 ± 2 vs. 13 ± 2 ml/min) changed significantly, but plasma glucose increased from 100 ± 3 to 167 ± 21 mg/dl, $P < .01$. The increases in plasma glucose during infusion of insulin with the Biostator and the Harvard pump were not significantly different ($t = 1.44$, $P = .19$). When insulin was delivered directly into test tubes with the Biostator and the Harvard pump, insulin concentrations in the test tubes decreased 46% over 8 h ($P < .05$) with the Biostator, whereas no decrease was observed with the Harvard pump. We conclude that the dawn phenomenon can occur without an increase in insulin clearance and that the apparent overnight increase in insulin clearance observed in studies with the Biostator is artifactual. DIABETES 1986; 35:749–52.

The amount of insulin necessary to maintain euglycemia increases between midnight and 0800 h in both nondiabetic[1,2] and diabetic[3–12] individuals, an occurrence commonly referred to as the dawn phenomenon.[13–16] The increases in plasma insulin and/or C-peptide levels observed in nondiabetic subjects[1,2] and patients with non-insulin-dependent diabetes[3] (NIDDM) during this period suggest that decreased insulin sensitivity rather than decreased insulin availability is primarily responsible. Several recent studies in patients with insulin-dependent diabetes (IDDM) have provided evidence that the decreased sensitivity to insulin is due to nocturnal surges in growth hormone secretion.[17–19]

Nevertheless, observations that plasma free-insulin levels decreased overnight during apparent constant intravenous infusion of insulin in patients with IDDM suggest that an increase in clearance of insulin may also be involved in the pathogenesis of the dawn phenomenon.[9,11,12,18] However, most studies[9,11,18] demonstrating an overnight decrease in plasma insulin concentrations used the Biostator (Miles Laboratories, Elkhart, IN)[20] to infuse insulin. This device has recently been shown to decrease its delivery of biologically and immunologically active insulin over time under conditions comparable to those used in these studies, thus raising the possibility that the apparent overnight increase in insulin clearance might be an artifact.[21,22]

Our study was undertaken to examine this issue further and to determine the contribution of this decrease in plasma insulin to the magnitude of the dawn phenomenon in patients with IDDM. For this purpose we compared insulin delivery by the Biostator and a Harvard pump (Harvard Apparatus, Millis, MA) over comparable 8-h intervals both in vitro and in subjects with IDDM (midnight to 0800 h). Our results demonstrate that insulin delivery decreases over time with the Biostator but not with a Harvard pump and that a substantial dawn phenomenon can occur in the absence of a decrease in plasma free insulin in patients with IDDM. Thus, previously observed overnight increases in insulin clearance in studies with the Biostator are probably artifactual and cannot account for the dawn phenomenon.

METHODS

In vitro experiments. For the in vitro assessment of insulin delivery, insulin (Iletin II, Lilly, Indianapolis, IN) was infused for 8 h at a constant rate of 13 mU/min into test tubes on a

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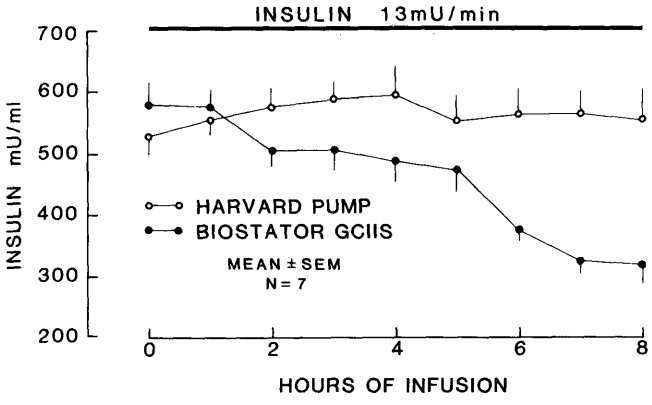

FIGURE 1. Changes in insulin concentrations in test tubes into which insulin was infused by Biostator or Harvard pump.

fraction collector with either the Biostator (N = 7) or a Harvard pump (N = 7). Each test tube collected infusate for 1 h. In the experiments with the Biostator, 300 U of insulin was added to 500 cm^3 of normal saline, and 200 cm^3 of this solution was discarded through the infusate tubing before starting the experiment. In the Harvard pump experiments, 30 U of insulin was added to 50 cm^3 of normal saline containing 1% human serum albumin (Travenol Laboratories, Glendale, CA). The infusion rates were 0.022 cm^3/min for both the Harvard pump and Biostator experiments. Insulin was measured by radioimmunoassay.[23]

In vivo experiments. For the in vivo assessment of insulin delivery, informed written consent was obtained from eight volunteers (6 men, 2 women) with IDDM (plasma C-peptide response to intravenous glucagon <0.1 ng/ml) aged 21–48 yr and within 15% of their ideal body weight.[24] All volunteers took part in two experiments performed in random order; each experiment was separated by at least 1 wk. Subjects were withdrawn from their intermediate-acting insulin at least 30 h before study and were subsequently managed with multiple subcutaneous injections of regular insulin as previously described.[10] No subcutaneous regular insulin was given within 12 h of starting the study. At 1700 h, subjects were placed at bed rest in the Clinical Study Unit, connected to a closed-loop insulin infusion device (Biostator GC IIS, Life Science Instruments, Miles Laboratories) and given a standard meal (600 kcal, 47% carbohydrate, 32% fat, and 21% protein). An arm vein contralateral to that connected to the closed-loop insulin infusion device was cannulated for blood sampling. Subjects were rendered euglycemic (90–110 mg/dl) within 4 h. The variable insulin infusion from the Biostator was stopped at midnight, and an infusion of insulin at a constant rate (0.15 mU · kg^{-1} · min, Iletin II) was started. In one experiment this insulin, dissolved in 0.9% saline containing 1% human serum albumin (Travenol Laboratories), was infused by a Harvard pump (Harvard Apparatus). In the other experiment the insulin was made for use as recommended by the manufacturer, similar to that used in previous studies demonstrating an overnight increase in insulin clearance (300 U insulin/500 cm^3 normal saline),[9,11,18] and was infused by the Biostator in the 7:1 constant-rate mode. Blood was drawn every 30 min for measurement of plasma free insulin[25] and glucose (YSI Glucose Analyzer) concentrations. All subjects slept between 2300 and 0700 h.

Data are given as mean ± SEM. Insulin clearance was calculated by dividing the insulin infusion rate by the plasma insulin concentration.[26] Statistical analyses were performed by use of paired Student's t tests.

RESULTS

In vitro insulin delivery. When the insulin was infused into test tubes with a Harvard pump, the insulin concentration in the test tubes did not change significantly over the 8-h infusion period (523 ± 27 vs. 558 ± 46 mU/ml, P = NS); in contrast, when the Biostator was used to infuse insulin, there was a 46% decrease in insulin concentration over this interval (585 ± 30 vs. 317 ± 35 mU/ml, $P < .05$; Figure 1).

In vivo insulin delivery. During infusion of insulin with the Harvard pump, plasma free-insulin concentrations did not change significantly from midnight to 0800 h (14 ± 1 vs. 13 ± 1 μU/ml, P = NS). Consequently, there was no change in insulin clearance over this period (12 ± 2 vs. 13 ± 2 ml/min, P = NS; Figure 2).

During infusion of insulin with the Biostator plasma free-insulin concentrations decreased nearly 40% from midnight to 0800 h (14 ± 1 vs. 9 ± 1 μU/ml, $P < .05$). This resulted in an apparent increase in insulin clearance from 11 ± 1 to 17 ± 2 ml/min, $P < .05$.

Plasma glucose concentration increased significantly both during Harvard pump and Biostator infusions of insulin. Although the plasma glucose concentration at 0800 h was greater when the insulin was infused with the Biostator than with the Harvard pump (217 ± 27 vs. 167 ± 21 mg/dl), this difference was not statistically significant (t = 1.44, P = .19).

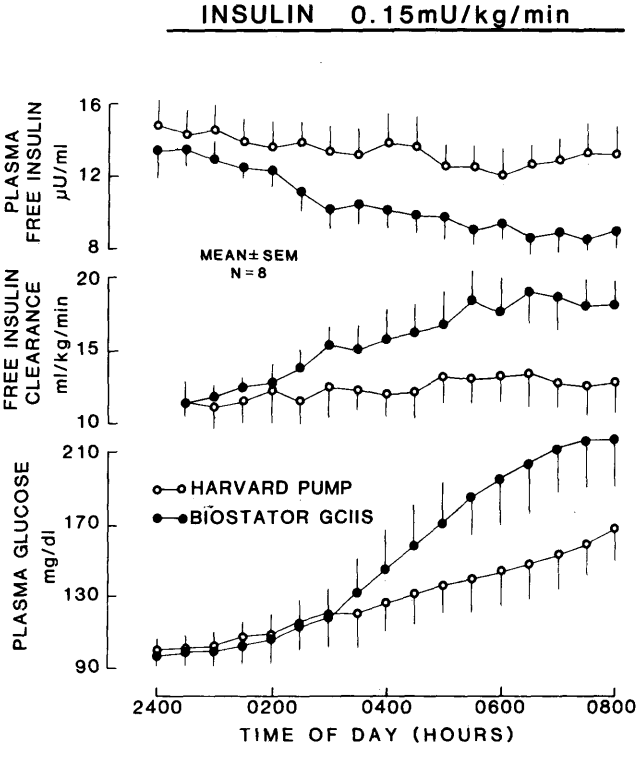

FIGURE 2. Plasma free-insulin concentrations, free-insulin clearance rates, and plasma glucose concentrations during infusion of insulin with Biostator or Harvard pump in subjects with IDDM.

DISCUSSION

Our in vitro studies indicate that the Biostator decreases its delivery of insulin over time, confirming two other recent reports.[21,22] Brennan et al.[21] provide evidence that this decrease is due to aggregation of the insulin molecules passing through the Biostator pump assembly and attribute it to heat generated by the pump. Insulin aggregation was prevented either by increasing the insulin solution flow rate to 16 ml/h[21] or by adding either 2% serum albumin[21] or heparinized whole blood[22] to the insulin solution. The 45% decrease in Biostator insulin delivery observed in our in vitro studies was similar to that observed in our in vivo studies with the Biostator and in the in vitro studies of Brennan et al.[21] but somewhat less than the 60–80% decrease observed in the in vitro studies of Harris et al.[22]

Several previous studies conducted in patients with IDDM found decreases in plasma insulin levels during the overnight period while subjects were receiving an apparently constant intravenous infusion of insulin.[9,11,12,18] Three of these studies used the Biostator to infuse the insulin.[9,12,18] In the only study that did not use a Biostator but found an increase in insulin clearance overnight,[11] the plasma free-insulin levels observed were nearly twofold greater than those expected from the insulin infusion rates used,[26–29] and insulin clearance increased only 8%. Moreover, there was no change in the amount of exogenous glucose required to maintain euglycemia, which would have been expected if insulin clearance had increased. Thus, the interpretation of these studies is open to question.

The results of our in vivo experiments with the Harvard pump, in which no change in the plasma free-insulin levels was observed, demonstrate that the dawn phenomenon can occur without an overnight increase in insulin clearance; in these studies plasma glucose increased ~70 mg/dl overnight. The impact of the reduced insulin delivery rate from the Biostator on the early morning rise in plasma glucose can be gauged by the 50 mg/dl difference in the plasma glucose concentration observed when the insulin was infused by either the Biostator or the Harvard pump (217 ± 28 vs. 167 ± 21 mg/dl at 0800 h, respectively, P = NS). Although this difference was not statistically significant, we consider it likely, from what is known about hepatic sensitivity to insulin, that the decrease in insulin availability of the magnitude observed in our study would produce a significant difference if additional subjects were studied.[28,29]

In our earlier study, which demonstrated a role for nocturnal surges in growth hormone secretion in the pathogenesis of the dawn phenomenon in IDDM patients,[18] plasma glucose increased by 40 mg/dl overnight during apparent constant intravenous infusion of insulin from the Biostator despite complete suppression of growth hormone secretion by a somatostatin infusion. In that study the plasma free-insulin concentration decreased ~4–6 μU/ml. Based on the results of the present experiments, it seems likely that the residual early morning hyperglycemia may be explained by the decrease in insulin delivery with the Biostator.

In conclusion, our results indicate that in patients with IDDM, insulin clearance does not change between midnight and 0800 and that plasma glucose concentrations increase during this period despite constant plasma free-insulin concentrations. Thus, an increase in insulin clearance is not responsible for the dawn phenomenon. Previous reports suggesting an overnight increase in insulin clearance appear to be explained by aggregation of insulin during Biostator infusion of insulin.[9,11,18]

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