Pulsatile Insulin Has Greater Hypoglycemic Effect Than Continuous Delivery

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SUMMARY

The relative hypoglycemic effects of pulsatile versus steadily infused insulin have been examined in six normal subjects in whom pancreatic insulin output was suppressed by somatostatin-14. Soluble insulin was infused continuously overnight on one occasion and on another occasion the same quantity was given in pulses of 2-min duration with a gap of 11 min. The mean plasma glucose concentrations were lower when pulsed insulin was given [mean for the last hour: 4.66 ± 0.08 mmol/L (±SEM) versus 5.53 ± 0.06 mmol/L (±SEM) for steady infusion], diverging significantly ($P < 0.05$ paired *t* test) 7 h after the start of the study. The specific binding of ^{125}I(A14)mono-iodo-insulin to monocytes was greater after pulsed insulin (2.9% with pulsed versus 2.4% with steadily infused insulin at tracer-only point; $P < 0.02$ paired *t* test). Thus, intravenous insulin has greater hypoglycemic effect when pulsed, possibly mediated by greater insulin receptor binding. DIABETES 32:617–621, July 1983.

Basal plasma insulin concentrations oscillate in man[1–3] and monkeys[4] with a periodicity of 14 min. The oscillations persist in response to stimuli such as glucose, with larger pulses providing the increased secretion.[5] Since these stable pulsations are not found in non-insulin-dependent diabetics,[5,6] their absence may contribute to the basal hyperglycemia. This is analogous to LHRH having a greater effect with pulsatile release than with steady levels.[7,8] Receptors become less sensitive to hormonal stimuli when exposed to high steady concentrations;[9,10] thus, oscillatory hormonal stimuli could help to maintain receptor integrity. The present study was undertaken to test the hypothesis that oscillatory insulin might have a greater hypoglycemic effect than continuous delivery in man.

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MATERIALS AND METHODS

Subjects and protocol. Nine normal subjects were recruited into the study. Three subjects had experiments terminated: one because of abdominal cramps, one because of a fever, and one because of a blood alcohol > 30 mg/dl. The remaining six normal subjects (five men and one woman, aged 21–34) were studied overnight on two occasions in random order. On both occasions somatostatin-14 (Serono Laboratories Ltd., United Kingdom) was infused continuously and intravenously from midnight until 9:00 a.m. at 100 μg/h to suppress endogenous insulin and glucagon production. Hepatic glucose output was maintained by glucagon replacement (12.5 μg/h) infused with the somatostatin. One hand was kept warm with an electric heating pad, and integrated heparinized blood samples (2 ml/15 min) were taken by continuous pump from a venous cannula inserted at the wrist. At 8:00 a.m., 110 ml of venous blood was taken for receptor binding studies and the integrated sampling rate increased to 2 ml/min for a final hour. Soluble insulin (Actrapid, Novo, Copenhagen, Denmark) was administered intravenously over one night as a continuous infusion at a rate of 8 mU/min, which was chosen as being near the lower end of the insulin delivery rate for normal subjects.[11] On the other night an identical amount of insulin in toto was given using pulses of 2-min duration followed by gaps of 11 min. Switching of the pump was achieved using an electronic timer. Insulin, glucagon, and somatostatin adsorption to plastic was minimized by diluting the hormones in 120 ml 0.154 M saline to which 0.2 g polygeline (Haemaccel:Hoechst) had been added. Blood samples were kept in iced water before centrifugation, and the plasma frozen within 3 h.

Assays. Plasma glucose was estimated by the glucose-oxidase method in a semiautomated system (Pye Unicam AC1, CV: 1.45%). Plasma insulin and C-peptide were assayed by charcoal-phase-separation radioimmunoassay[12,13] (CV: 13.7% and 9.1%, respectively). The erythrocyte separation and monocyte receptor assays were carried out using a modified method of Gambhir[14] previously described by Ward et al.[15] using ^{125}I(Tyr A14)insulin (Novo), 100 pg/tube for monocytes and 70 pg/tube for erythrocytes.

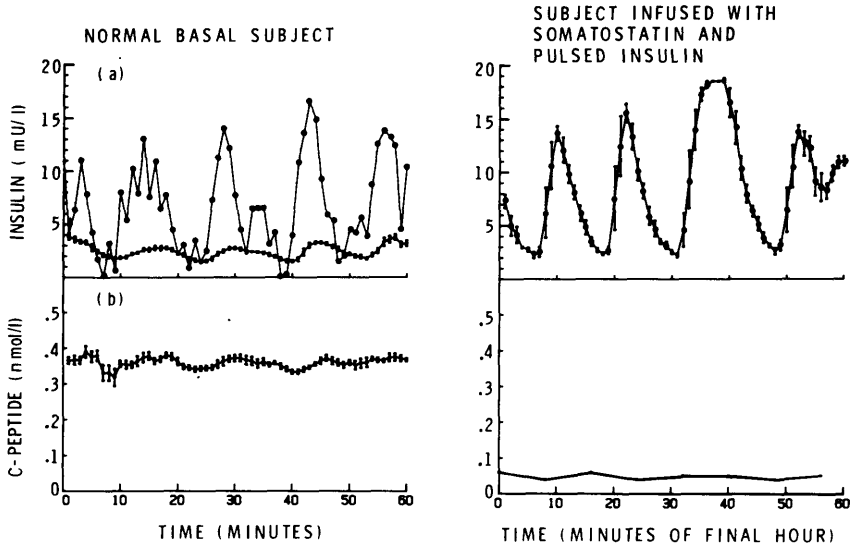

FIGURE 1. Comparisons of plasma insulin and C-peptide concentrations in normal basal subject (left) and normal subject after an overnight fast infused with somatostatin and pulsatile insulin (right). Upper panels: insulin concentrations (shown as points with bars) of a normal and experimental subject. Superimposed on the normal subject's concentration profile is the portal concentration (●) estimated using deconvolution analysis,[11] assuming the blood flow to the liver is 25% of the cardiac output[31] and that 50% of the insulin is extracted by the liver.[32] This demonstrates a similarity between the hepatic concentrations of insulin in the normal and experimental situation. Lower panels: C-peptide concentrations. C-peptide shows concordance with the insulin concentrations in the normal subject (left), while in the experimental subject the low concentrations reflect suppression of endogenous insulin. Bars: ±SEM of 3-min moving average.

The monocyte fractions were isolated from 100 ml of whole blood taken into 1000 U heparin. Stock isotonic percoll was prepared from Percoll (Pharmacia) by mixing 9 vol with 1 vol 0.23 NaCl and diluting to a density of exactly 1.077 g/ml with Tris saline. This was pumped at a rate of 1 ml/min under 33 ml of whole blood in each of 3 × 50-ml polycarbonate tubes. The tubes were centrifuged at 800 × *g* (at cell interface) for 30 min at room temperature. After the supernatant was discarded, the mononuclear cell layer was aspirated from each tube and resuspended in 20 ml Tris saline. This was centrifuged at 100 × *g* for 15 min at 0°C. The cells were washed and made up to a concentration of not less than 25 × 10^7/ml in monocyte buffer.[16] The cells were checked for viability using trypan blue (for all suspensions viability > 95%). Monocytes were identified by nonspecific esterase staining.[17] The percentage of monocytes ranged from 12% to 28%, the other cells being almost exclusively lymphocytes identified by Leishman's stain. The same mono-iodinated ^{125}I(A14)insulin was used on each of the occasions for each subject.

Statistical testing used paired *t* tests. Calculations were carried out on a CTL computer. Subjects gave informed consent and these experiments were passed by the Oxford Area Health Authority (T) Ethics Committee.

RESULTS

C-peptide and insulin. Figure 1 shows that the measured peripheral insulin concentrations during the final hour of pulsed insulin infusion are similar to the estimated pulsatile portal vein insulin levels of a normal subject. This figure also shows that in the normal basal state the C-peptide oscillations reflect the insulin oscillations with a smaller amplitude consequent on the longer half-life of C-peptide, whereas in

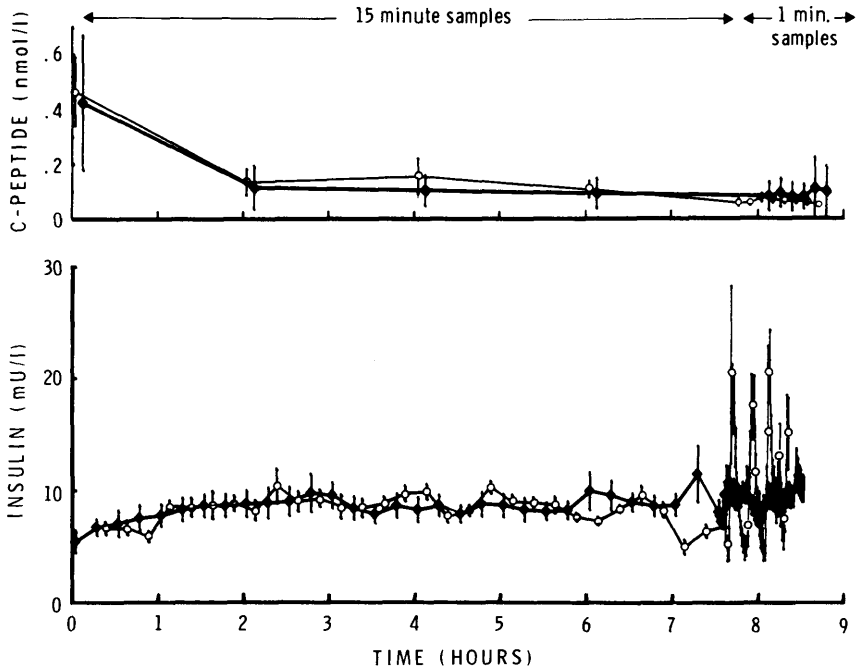

FIGURE 2. Mean C-peptide and insulin concentration profiles throughout the experimental period for six subjects. Thick lines (◆) represent data from the steady-state insulin infusion experiments, and thin lines (○) data from the pulsed insulin experiments. Pulsations do not show on the first 7.5 h overnight because each sample was continuously withdrawn (integrated) over 15 min during this period. For the last hour, sampling was changed to every minute, and pulsations are consequently apparent between 5 and 19 mU/L for pulsed insulin, with little variation around 9 mU/L for the steadily infused insulin. Bars: ±SEM.

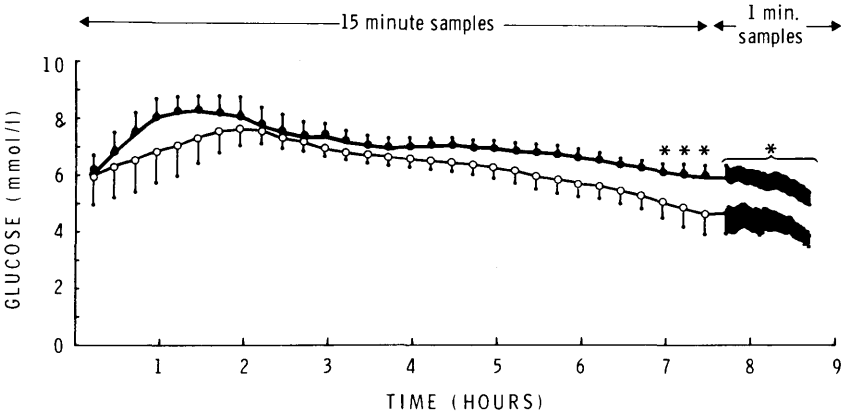

FIGURE 3. Mean plasma glucose concentrations throughout the experimental period for six subjects. Thick line (●) represents data from steady insulin infusion, and thin line (○) represents data from pulsed insulin experiments. Significance is shown by *: $P < 0.05$, paired *t* test. Bars: SEM.

the experimental subject the C-peptide is suppressed by somatostatin.

The mean data for all subjects showed adequate suppression of endogenous insulin secretion, since the C-peptide concentrations fell from a mean of 0.42 to 0.09 nmol/L for steady insulin infusions and from 0.46 to 0.06 nmol/L for pulsed insulin infusions (Figure 2). The overall insulin concentrations measured on the integrated samples were not significantly different throughout the duration of the study (Figure 2), but the mean peripheral plasma insulin concentration was higher than the normal basal concentration.

Glucose. Initially plasma glucose concentrations were similar on both occasions (mean 4.19 mmol/L before steady insulin infusion and mean 4.42 mmol/L before pulsed insulin infusion). The concentrations became higher on the occasion when insulin was infused steadily [mean for the last hour: 5.53 ± 0.06 mmol/L (±SEM)] than when insulin was delivered in pulses [mean for the last hour: 4.66 ± 0.08 mmol/L (±SEM); Student's paired *t* test $P < 0.05$]. Overnight the mean glucose concentrations significantly diverged after 7 h of infusion of insulin (Figure 3).

Insulin receptors. The total specific binding of ^{125}I(A14)mono-iodo-insulin to monocytes was greater after overnight pulsed insulin than after continuously infused insulin (Figure 4) ($P < 0.02$ by Student's *t* test for paired samples at labeled insulin-only point). Analysis of the data assuming separate high- and low-affinity binding sites[18] suggests an increase in affinity for both classes of receptor when insulin is delivered in pulses. (Figure 5 shows the curve-striping deconvolution.) Analysis according to a negative-cooperativity hypothesis[19] suggests an average increase of 50% in the affinity constants for both empty and full sites after pulsatile insulin (K_e 13.5 ± 2.4 to 20 ± 5.8 and K_f 3.0 ± 0.6 to 4.9 ± 1.5 $M^{-1}/10^8$). There was no change in erythrocyte insulin binding between the experiments.

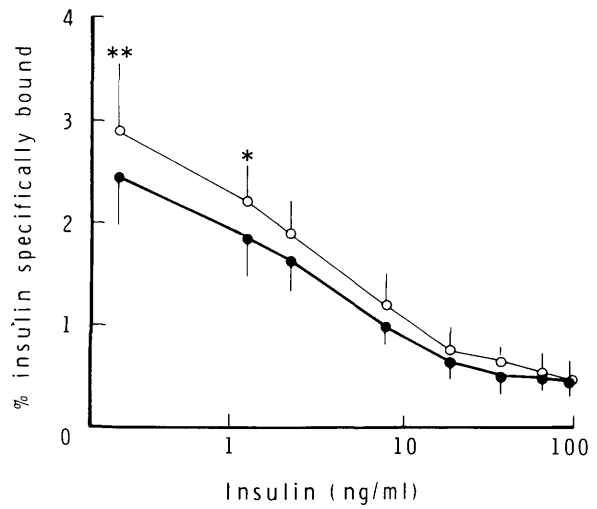

FIGURE 4. Mean specific binding of ^{125}I(A14)mono-iodo-insulin to monocytes for six subjects. Thick line (●): after continuous insulin infusion. Thin line (○): after pulsatile insulin. Significance is shown by **: $P < 0.02$, *: $P < 0.05$ paired *t* test. Bars: SEM.

DISCUSSION

Oscillatory insulin production in man has not previously been shown to have metabolic implications, but this study suggests that in normal subjects infusion of insulin in pulses has a greater glucose-lowering effect than continuous insulin delivery. Monocyte receptors showed a relatively enhanced insulin binding with pulsatile insulin or a relative downregulation with continuous insulin. Thus, the lower plasma glucose concentration with pulsed insulin could, in part, be explained on the basis of receptor function. Nevertheless, a concordant change does not necessarily imply a causative effect. The erythrocyte insulin receptor binding data did not change, but this may reflect the different receptor characteristics between monocytes and erythrocytes.[20] Other mechanisms may also be affected by different insulin delivery profiles. Evidence from rhesus monkeys suggests that alternating hepatic glucose efflux and influx is entrained by regular insulin oscillations,[21] and this may allow an appropriate hepatic response to any homeostatic demand. Insulin was infused in a peripheral vein in these experiments; however, in order to achieve physiologic insulin concentrations in the portal vein, higher peripheral insulin concentrations than normal were produced. The subjects were still normoglycemic after 7 h of insulin infusion; this finding lends support to the hypothesis that, in the basal state, control of hepatic gluconeogenesis is a major determinant of the glucose concentration. Intermittent insulin administration with peaks of insulin concentration may enhance peripheral glucose utilization or suppression of gluconeogenesis.

Physiologic oscillation of hormone production is now well documented. It is known to occur with insulin in baboons,[22] monkeys,[4,23] and dogs;[24] vasopressin has episodic secre-

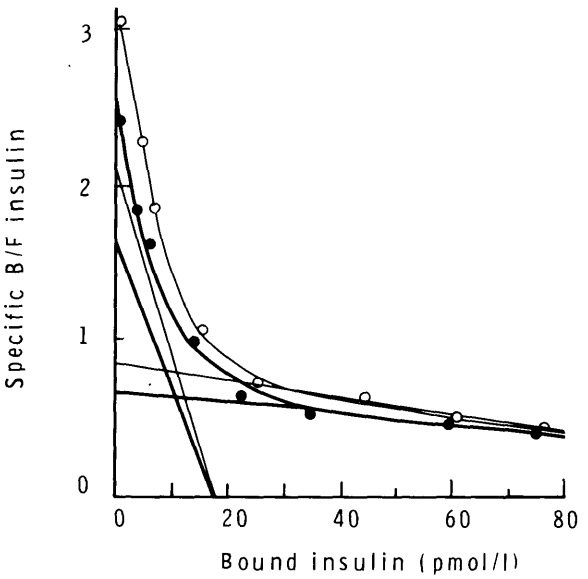

FIGURE 5. Scatchard plot of insulin-binding curves. The specifically bound:free ratio (× 100) is plotted against bound insulin. Thick lines (●): data for steady insulin infusion experiments. Thin lines (○): data for pulsed insulin infusion experiments. Straight lines show the deconvoluted analysis into high- and low-affinity receptor binding, with an apparent change in insulin receptor affinity.

tion;[25] LHRH oscillations occur with the onset of puberty in boys;[26] and LH can be demonstrated to have a phasic release pattern.[27] Steady-state levels can reduce responsiveness,[8] and hypophyseal responses are optimized by intermittent rather than continuous delivery of LHRH.[7] This has been used clinically in the treatment of Kallman's syndrome[28] and anorexia nervosa.[29]

Receptor regulation of target cells' responsiveness to hormone has been shown for insulin, catecholamines, gonadotropins, angiotensin II, somatostatin, and TRH.[30] So-called downregulation at the cellular level may partially explain the paucity of action of steady-state levels, while pulsatile hormone may allow recovery of receptor affinity or numbers. Insulin receptors have been demonstrated to downregulate over a matter of hours when exposed to high steady-state insulin concentrations in vitro[9] and in vivo.[10] These findings are in accord with the authors' in vivo observation using insulin at near-normal concentrations. By analogy with such experiments, it is possible that steadily infused insulin relatively downregulated monocyte insulin receptors, while mimicking basal insulin pulsations preserved their normal sensitivity; however, we do not have receptor data from a control day to substantiate this finding. Quite apart from the pulsatility of secretion enhancing the effect of insulin, discrete secretory pulses probably allow increased sensitivity of control of basal insulin secretion and improve basal glucose homeostasis.[5]

The enhanced effect of pulsatile insulin secretion may have clinical implications. Since oscillatory insulin has greater hypoglycemic actions than steadily infused insulin in normal subjects, the effects of intravenous insulin given by pumps to diabetics might be augmented by pulsations. Subcutaneous administration of insulin achieves steady or slowly changing insulin concentrations in plasma, and lack of pulsatility may mean suboptimal action, quite apart from other factors such as the peripheral rather than portal route of administration and difficulties with matching doses to meal size. Since non-insulin-dependent diabetic subjects do not have the regular insulin oscillations[5] found in normal subjects,[1] part of their insulin resistance might be attributable to deranged temporal quality, as opposed to total quantity, of insulin production.

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