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Rapid communication

Basal activity profiles of NPH and [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulins in subjects with IDDM

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Summary [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin is a fatty acid-acylated derivative of insulin with extended action compared to unmodified insulin when infused intravenously (i.v.) secondary to its binding to circulating albumin. The duration and activity profile of the acylated (A) and NPH (B) insulins were assessed following subcutaneous (s.c.) doses of (A) 6 nmol/kg and (B) 1.2 nmol/kg (equivalent to 0.2 U/kg) in 9 subjects with IDDM. After overnight i.v infusion of regular human insulin, morning glucose was (A) 6.9 ± 0.1 and (B) 6.8 ± 0.1 mmol/l. After the s.c. injection, i.v. human insulin or glucose was infused to maintain near-basal glycaemia and tracer glucose to assess hepatic glucose production (HGP). An activity profile was deduced for each study by expressing the glucose infusion rate at each time point, as a fraction (%) of the basal (measured) HGP, and the i.v. insulin infusion rate as a fraction (%) of the basal requirement. The two fractions are combined by adding the fractional glucose infusion rate and subtracting the fractional insulin infusion rate. Infusion rates of i.v. insulin in the morning were (A) 0.96 ± 0.096 and (B) 1.22 ± 0.09 pmol $\cdot$ kg^{-1} $\cdot$ min^{-1}. After insulin injection, i.v insulin requirements decreased and were below 10% of basal between 100 and 150 min. A constant activity profile of 0% represents a perfect substitution of the basal i.v. insulin infusion by the s.c. dose. The actual profile is defined by deviations from this (above) and was -17 ± 11, 7 ± 10, -9 ± 6 and -18 ± 18% for [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin and 17 ± 12, 5 ± 6, -9 ± 15, 22 ± 18% for NPH insulin at 3, 6, 9 and 12 h after s.c. injection. HGP was similar for the two insulins, demonstrating similar metabolic actions and profiles both peripherally and at the liver. [Diabetologia (1998) 41: 116--120]

Intensive insulin therapy in insulin-dependent diabetes mellitus (IDDM) has been shown to reduce the incidence of microvascular complications [1]. Postprandial glucose excursions have recently been specifically targeted in the development of rapid-acting insulin analogues [2--4]. The complementary problem is the optimization of basal insulin therapy. Control of basal glycaemia is generally achieved using intermediate acting insulins such as Neutral Protamine Hagedorn (NPH) or Lente preparations which have a protracted action based on interactions of insulin with protamine, preservatives and/or zinc. The insoluble nature of these preparations may however lead to variability both in their administration and their absorption [5, 6].

An alternative strategy, recently introduced, extends the action of insulin by inducing its binding to endogenous proteins [7,8]. This was achieved by fatty acid acylation of the insulin and the binding of the fatty acyl insulin to albumin, leading to extension of action by increasing the residence time at the site of injection, in the circulation, and in the interstitial space at the target sites. In vivo assessments of the action of [N$^{\varepsilon}$-myristoyl Lys (B29) des (B30)] human in-

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; NPH, Neutral Protamine Hagedorn

sulin [7] and [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin [8] have demonstrated the effectiveness of these preparations in animal models.

The primary goal of this work was to assess the action of [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin in humans. The strategy adopted was (i) to utilize the target population (subjects with IDDM) and basal conditions established by an overnight intravenous infusion of regular insulin; (ii) to determine rates of metabolic clearance (MRC) and production of glucose (R_a) so as to differentiate between peripheral and hepatic action of the acylated insulin; (iii) to utilize this information in the development of an activity profile for the new insulin and (iv) to compare its action to that of NPH human insulin.

Subjects and methods

Nine patients, 18 years of age or over with C-peptide negative IDDM of more than 2 and less than 15 years duration and acceptable metabolic control participated in these studies. All patients were managed with appropriate combinations of regular and intermediate-acting (e. g. NPH) insulins. The study was approved by the Human Ethics Committee of the Ottawa Civic Hospital and all subjects gave their informed consent. Each subject received in random order and at least 1 week apart, a subcutaneous injection of [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin and of Humulin N.

Each patient was managed with preprandial regular insulin only for 24–36 h prior to the study, was admitted on the afternoon before and placed on i. v. insulin and glucose monitoring following the evening meal. A sliding scale for the infusion was used to achieve morning near-normoglycaemia [9]. At t = --150 min an infusion of either [6-^{3}H]- or [1-^{14}C] glucose was initiated. At t = 0 min, a s. c. injection of either NPH (1.2 nmol/kg) or [N$^{\varepsilon}$-palmitoyl Lys (B29)] (6 nmol/kg) human insulin was administered. Plasma glucose was monitored every 5--10 min throughout the study. Initially ($t < 0$) the insulin infusion was adjusted to maintain basal glycaemia. After subcutaneous insulin injection, the i. v. insulin infusion was tapered so as to maintain basal glycaemia. After discontinuation of this infusion, a 20 % glucose solution was added to the infusion line and either glucose or insulin was administered if necessary to maintain basal glucose levels. The study continued until t = 780 min and samples were taken for tracer, glucose, lactate and insulin levels at 10 to 30 min intervals, and hourly for NEFA and β-hydroxybutyrate.

Materials. Administered substances include: Humulin N which is human insulin [recombinant DNA origin] in isophane suspension at a concentration of 100 U/ml (600 nmol/ml). The acylated insulin is [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin (rDNA), (600 nmol/ml), both obtained from Eli Lilly and Co. (Indianapolis, Ind., USA). [6-^{3}H] glucose and [1-^{14}C] glucose were from Amersham Corporation, Arlington Heights, Illinois, USA) and were purified using ion-exchange HPLC (Column: HPX-87P; Biorad, Hercules, California, USA) passed through a Gelman Acrodisc filter (0.2 μ: Ann Arbor, Michigan, USA) and tested for sterility and pyrogenicity.

Analytical methods Plasma glucose, tracer, lactate and insulin were determined as previously described [4]. Levels of total [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin were determined using a rat insulin antibody (Linco Research, St. Charles, Missouri, USA). Standard curves were prepared using the acylated insulin and separation of bound and free was achieved using a second antibody (Linco Research). The intra-assay coefficient of variation was ± 3 %.

Calculations. The MCR and glucose production rates (R_a) were calculated using a two-compartment description of glucose kinetics. The endogenous glucose production is determined by subtracting the glucose infusion rate from R_a. An activity profile for the subcutaneously injected intermediate-acting insulins was then determined as follows. Any insulin requirement after the s. c. injection is defined as a fraction of the basal ($t < 0$) insulin infusion rate. Any glucose requirement is defined as a fraction of the basal glucose production rate (R_a). Since the insulin and glucose infusions act in opposite directions on glycaemia and since they are never administered simultaneously, the fractional insulin infusion can be considered negative and the glucose infusion positive. Adding the two together then yields a continuous profile corresponding to the activity of the injected insulin under basal conditions or, the context in which a basal insulin must act. Clearly this is 0 % when the injected insulin maintains basal glycaemia; it is negative when the depot insulin is insufficient for this and positive (glucose added) when there is more depot insulin absorbed than is necessary to maintain the basal turnover of glucose.

Statistical analysis. Analysis of variance with time as a repeated measure was used to compare the time-courses of concentrations or rates. Profiles were also compared using covariance analysis and the assumption of antedependence and a chi-square statistic. The data are presented as means ± SEM.

Results

Subject characteristics. Nine subjects with C-peptide negative IDDM participated in these studies. The mean age was 31 ± 3 years (range 21--42 years) and the mean duration of diabetes was 10.2 ± 1.1 years (range 5--15 years). Body mass index was 24.6 ± 0.6 (range 21.0--27.6 kg/m^2) and HbA_{1c} levels were 8.6 ± 0.5 %.

Metabolic parameters. Overnight intravenous insulin infusion resulted in morning plasma glucose concentrations of 6.9 ± 0.2 and 6.8 ± 0.2 mmol/l in the case of [N$^{\varepsilon}$-palmitoyl Lys (B29)] and NPH human insulins, respectively. Following injection, glucose concentrations remained the same for $t < 540$ min, following which there was some divergence with final levels at 8.2 ± 0.4 for [N$^{\varepsilon}$-palmitoyl Lys (B29)] and 6.3 ± 0.5 nmol/l for NPH insulins (p = 0.03). Lactate remained unchanged. NEFA doubled and β-hydroxybutyrate quadrupled during the course of the study but there was no difference between the time courses for the two insulins.

Pharmacodynamics Overnight intravenous insulin infusion normalized basal glycaemia. Infusion rates in the morning were 165 ± 16 and 209 ± 16 μU · kg^{-1} · min^{-1} (0.961 ± 0.09 and 1.22 ± 0.09 pmol · kg^{-1} · min^{-1}

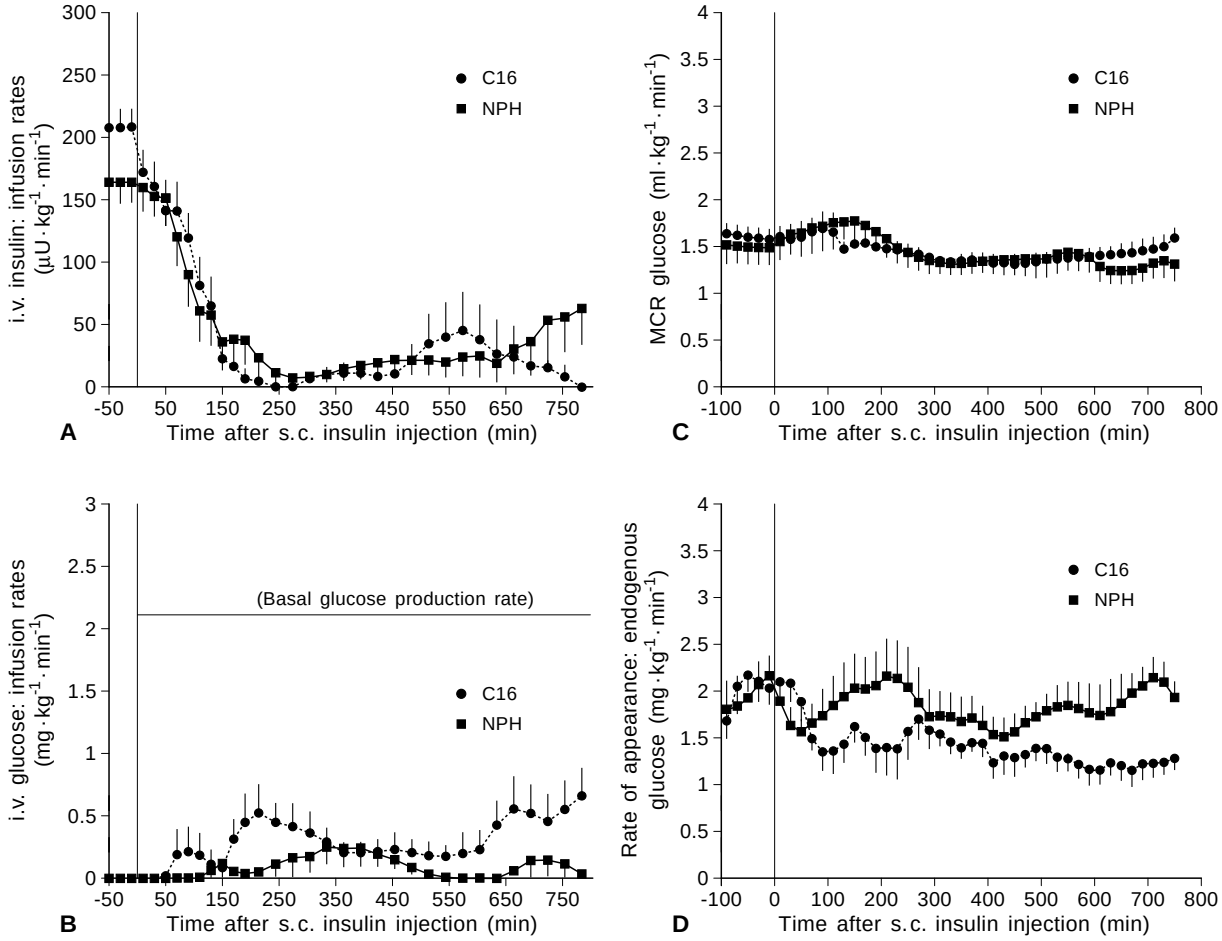

Fig. 1A--D. The infusion rates of intravenous insulin **(A)** and glucose **(B)** prior to (t < 0 min) and following the subcutaneous injection of 6 nmol/kg of [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin (C16) and 1.2 nmol/kg of NPH human insulin. Intravenous insulin infusion rates were adjusted along with glucose infusion rates in order to maintain a near normoglycaemia. Tracer-determined rates of the metabolic clearance of glucose (glucose MCR) **(C)** and endogenous (liver) production of glucose (R_a) **(D)** in the same experiment

for the studies where [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin and NPH human insulin were injected (Fig. 1A). Following injection of the two insulins, i.v. insulin requirements decreased and were below 10% of basal requirements between 100 and 150 min. Subsequently, mean requirements remained below 30% of basal. Mean infusion rates and infusion patterns were not different ($p > 0.05$). Similarly, neither were mean glucose infusion rates, or infusion profiles (Fig. 1B) different following the injection of either insulin ($p > 0.05$), although there is a suggestion that overall requirements were slightly higher following injection of NPH insulin. The MCR of glucose (Fig. 1C) demonstrated no difference in mean value ($p = 0.8$) or profile ($p = 0.7$) for the two insulins. The endogenous glucose production also remained essentially unchanged from basal (~ 2 mg · kg^{-1} · min^{-1}), did not differ following the two insulin injections either in the mean (1.8 ± 0.3 and 1.4 ± 0.2 mg · kg^{-1} · min^{-1} for [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin and NPH insulins, respectively, $p = 0.2$) or in its shape.

The immunoreactive level of insulin following [N^{3}-palmitoyl Lys (B29)] human insulin injection represents the total level (bound and free) and therefore reflects the circulating depot of this insulin. The maximum concentration was 6.23 ± 0.82 nmol/l at 297 ± 30 min after injection. The activity profile reflects the concentrations (-17 ± 11, -7 ± 10, -9 ± 6 and -18 ± 18.9% at 3, 6, 9 and 12 h) with a maximum effect reached at 254 ± 24 min.

Following NPH insulin the concentration is nearly constant and includes the contributions of both s.c. and i.v. insulin. The activity profile (-17 ± 12, 5 ± 6, -9 ± 15 and 22 ± 18% at 3, 6, 9 and 12 h) does not correspond to the concentration but rather to the effect of the depot insulin alone. It is slightly greater than that for [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin ($p = 0.03$) but this difference does not evolve in time ($p = 0.16$) nor are the patterns after the two injections different ($p = 0.9$). The maximal effect after NPH insulin was achieved at 203 ± 24 min, not different from the acylated insulin.

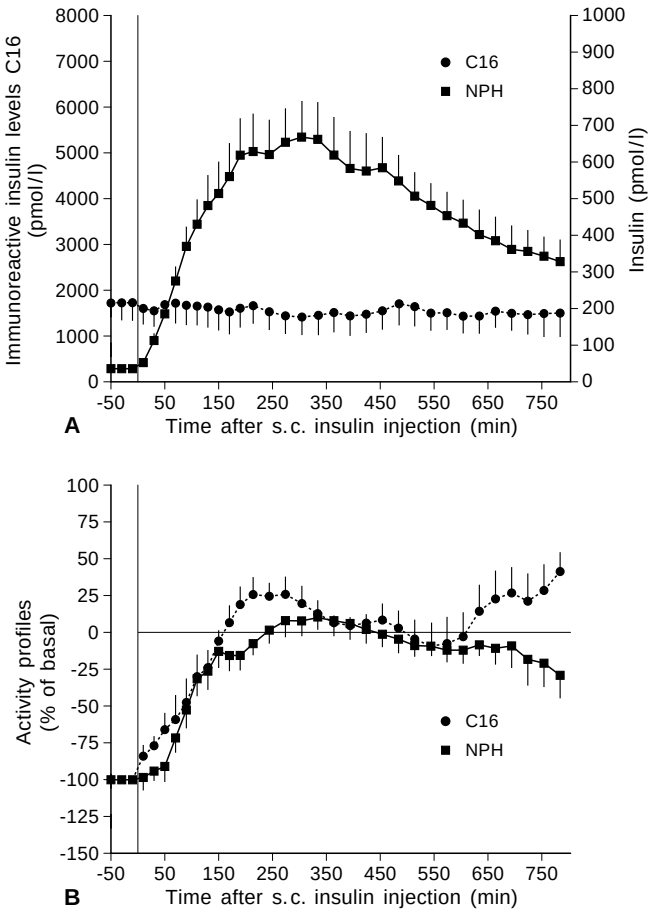

Fig. 2. A Plasma concentrations of immunoreactive insulin following subcutaneous injection of 6 nmol/kg of [N$^{\varepsilon}$-palmitoyl Lys (B29] human insulin (C16) or 1.2 nmol/kg of NPH human insulin. The levels of [N$^{\varepsilon}$-palmitoyl Lys (B29] human insulin include both the free and the bound forms of which most is the latter. **B** Activity profiles following the subcutaneous injection of the two insulins. The activity profile is defined as the i.v. glucose infusion rate (expressed as a % of basal hepatic production) minus the insulin infusion rate (expressed as % of the basal i.v. infusion rate)

Discussion

Although the dose was five times higher than NPH, [N$^{\varepsilon}$-palmitoyl Lys(B29)] human insulin demonstrated a similar extension of action to that for NPH insulin. The difference in dose could be due to differences either in potency or in availability. In the context of an insulin that circulates primarily in a bound form, availability would include mobilization from the injection site as well as from circulating and interstitial albumin. Differential availability for removal at the liver compared to action at target sites could also alter the apparent potency of such an insulin without any change in the activity of the free molecule. This is less likely than changes in absorption since (unpublished) data from this laboratory in swine suggest equivalent overall action of [N$^{\varepsilon}$-palmitoyl Lys (B29)] and regular human insulins, when this is administered intravenously.

NPH has been used extensively as a basal insulin. Extension of action is induced by crystallization with protamine and insulin and is administered as a suspension. It has been suggested that 80 % of the variation in its therapeutic effect is due to variability in absorption from the subcutaneous site [6]. Fatty acyl insulins are soluble and their action is extended by binding to albumin at the site of injection, in the circulation as well as in the interstitial space. The purpose of these studies was the assessment of the action of one such fatty acyl insulin, [N$^{\varepsilon}$-palmitoyl Lys (B29)] in humans, primarily with respect to its basal action. At basal conditions insulin action appears non-linear [10] with decreases from fasting insulin leading to non-parallel changes in the MCR of glucose and its production rate. These, in turn, are not simply the inverse changes to those which would be expected as insulin levels increase [10]. It was desirable therefore to examine the action of the acylated insulin near the physiological set point -- the set point which it is meant to maintain. C-peptide negative subjects with IDDM were therefore chosen.

Intravenous insulin infusion maintained this set point, or basal glucose turnover very well with basal turnover at 1.5--2 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} or very close to that seen physiologically in non-diabetic individuals. The activity profile (Fig. 2B) demonstrates that [N$^{\varepsilon}$-palmitoyl Lys (B29)] turnover insulin could maintain near-basal glycaemia for approximately 13 h. The profile of the activity of this acylated insulin was also similar to that of NPH, determined in the same subjects, as was the effect on glucose turnover. In the course of these studies, it also became evident that under these conditions, NPH insulin demonstrated less variability and more extension of action than generally thought.

In summary therefore, [N$^{\varepsilon}$-palmitoyl Lys (B29)] human insulin, an example of an acylated insulin which binds to albumin at the site of injection and in the circulation, is active under basal conditions in patients with IDDM, although the dosage is greater than that for NPH insulin. It is cleared more slowly and would thus be expected to be more available to the entire liver. Nevertheless it does not exhibit any differential effects in its action on the liver. Soluble insulins modified to bind to circulating proteins may, therefore, with development, offer an alternative as a basal insulin.

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