Preliminary Communications

HUMAN INSULIN PRODUCED BY RECOMBINANT DNA TECHNOLOGY: SAFETY AND HYPOGLYCÆMIC POTENCY IN HEALTHY MEN

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Summary Human insulin synthesised by recombinant DNA technology was compared with highly purified porcine insulin in healthy men. Intracutaneous injection over a wide range of concentrations of both insulins into five subjects gave rise to no local reactions over a 48 h period. The glycæmic response to standard subcutaneous injection at high and low dose levels was measured with both insulins in each of six men. Plasma glucose decrement with the two insulins was similar but human insulin was perhaps slightly more potent than porcine insulin at the low dose, and slightly less so at the high. The glycæmic response to the insulins, each infused intravenously at high and low concentrations for 1 h in a further six subjects, showed a similar trend. Depression of glycæmia with human insulin slightly exceeded that with porcine insulin at the low concentration infusion and fell slightly short of it at the high. Genetically synthesised human insulin seems to be safe and effective in man. Its dose-response relationship may differ from that of porcine insulin.

INTRODUCTION

THE physical and chemical properties of human insulin produced by recombinant DNA methods using genetically modified strains of *Escherichia coli* have recently been described and certain of its biological actions investigated in animals.[1] The first administration of this human insulin to man is described in this paper which reports on local reactions to intracutaneous injections and examines the glycæmic response to subcutaneous and intravenous administration. These responses are compared with those to highly purified porcine insulin of pancreatic origin.

SUBJECTS MATERIALS, AND METHODS

Seventeen healthy men aged between 24 and 56 years volunteered to take part in this experiment after full explanation. They were separated into groups for the three experiments described below (A, 5 subjects; B, 6 subjects; C, 6 subjects). They had never before received insulin injections and had no history of major or metabolic illness, atopy, or allergy. All subjects were within 20% of their ideal body-weight.[2] Diabetes mellitus and impaired glucose tolerance were excluded by formal testing.[3] The Guy's Hospital Ethical Committee gave permission for the experiments.

The A and B chains of human insulin were synthesised in the laboratories of Eli Lilly by separate strains of *E. coli*. These were genetically modified by the introduction of plasmids incorporating chemically constructed genes for each chain of human insulin as described by Goeddel et al.,[4] adapted in respect of scale, isolation, and purification techniques and improved peptide chain combination methodology. Chemical, toxicological, and immunological studies on the product (lot no. 46L35) in animals showed very high purity; in particular they failed to demonstrate, by any of several sensitive tests, the presence of abnormal forms of insulin or immunologically significant amounts of *E. coli* proteins (R. S. Baker and J. R. Schmidtke, personal communication). Specially purified porcine insulin (batch no. K81-156/615-075 256) was prepared in the Eli Lilly Research Laboratories and was constituted for use in these experiments at concentrations of 20 and 40 units/ml. The protein content of the highly purified porcine insulin was accurately measured and the human insulin was made up to a similar protein concentration. Both preparations were dissolved in the same standard diluent at pH 7·3.

A. *Skin Sensitivity Tests*

The five subjects in series A experiments were used to test for cutaneous hypersensitivity. A single test dose of 0·4 μg of the human insulin was first injected as a hypersensitivity screen. There were no positive responses five minutes later. Five equidistant sites were then selected between the wrist and the antecubital fossa on each arm and at each site 0·05 ml of solution was injected intracutaneously with a Mantoux syringe and 25 G needle. Eight insulin solutions were made up in diluent and contained human or purified porcine insulin 0·04 μg; 0·4 μg; 4 μg; and 40 μg. Insulin solvent and 0·154 mol/l sodium chloride solution were used as controls. Injection sites were randomised. Responses were read by one observer, ignorant of the randomisation, 5 min, 30 min, 5 h, 24 h, and 48 h after injection, and recorded by tracing through thin sheets of cellophane laid on the forearm.

B. *Subcutaneous Insulin Injections*

The six subjects in series B experiments fasted overnight before each of their four visits. A 'Teflon' venous sampling cannula was inserted into an antecubital vein and kept patent by a saline drip. Venous samples were withdrawn at time points illustrated in the accompanying figure, a, b. At between 08.30 and 09.00 h, 0·24 ml of human or porcine insulin was injected in random order into a different quadrant of the abdomen by the subcutaneous technique recommended by the British Diabetic Association. The subjects received either 4·8 or 9·6 units of purified porcine insulin or human insulin of the same protein concentrations. In each subject there was at least one day between experiments. The experiment was terminated with a carbohydrate-rich meal 5 h after injection. There was neither clinical nor biochemcial (plasma glucose <2·0 mmol/l) evidence of hypoglycæmia necessitating termination of the experiment.

C. *Intravenous Insulin Infusions*

The six other men were prepared similarly but received insulin through a cannula inserted into a vein in the non-sampling arm. To prevent insulin adsorption to the plastic infusion system, 7 ml of plasma from each subject was prepared under sterile conditions and added to a 500 ml bag of 0·154 mol/l sodium chloride solution. The insulin was then injected into

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the bag. (Experiments with ^{125}I-labelled insulin confirmed the prevention of adsorption by this technique.) Insulin solutions were infused for 1 h with an IVAC 630 piston pump at a volumetric rate of 50 ml/h delivering either 1·0 or 1·7 units/h of purified porcine insulin or the human insulin protein equivalents, in randomised order. Venous blood samples were collected at the intervals illustrated in the figure, c, d.

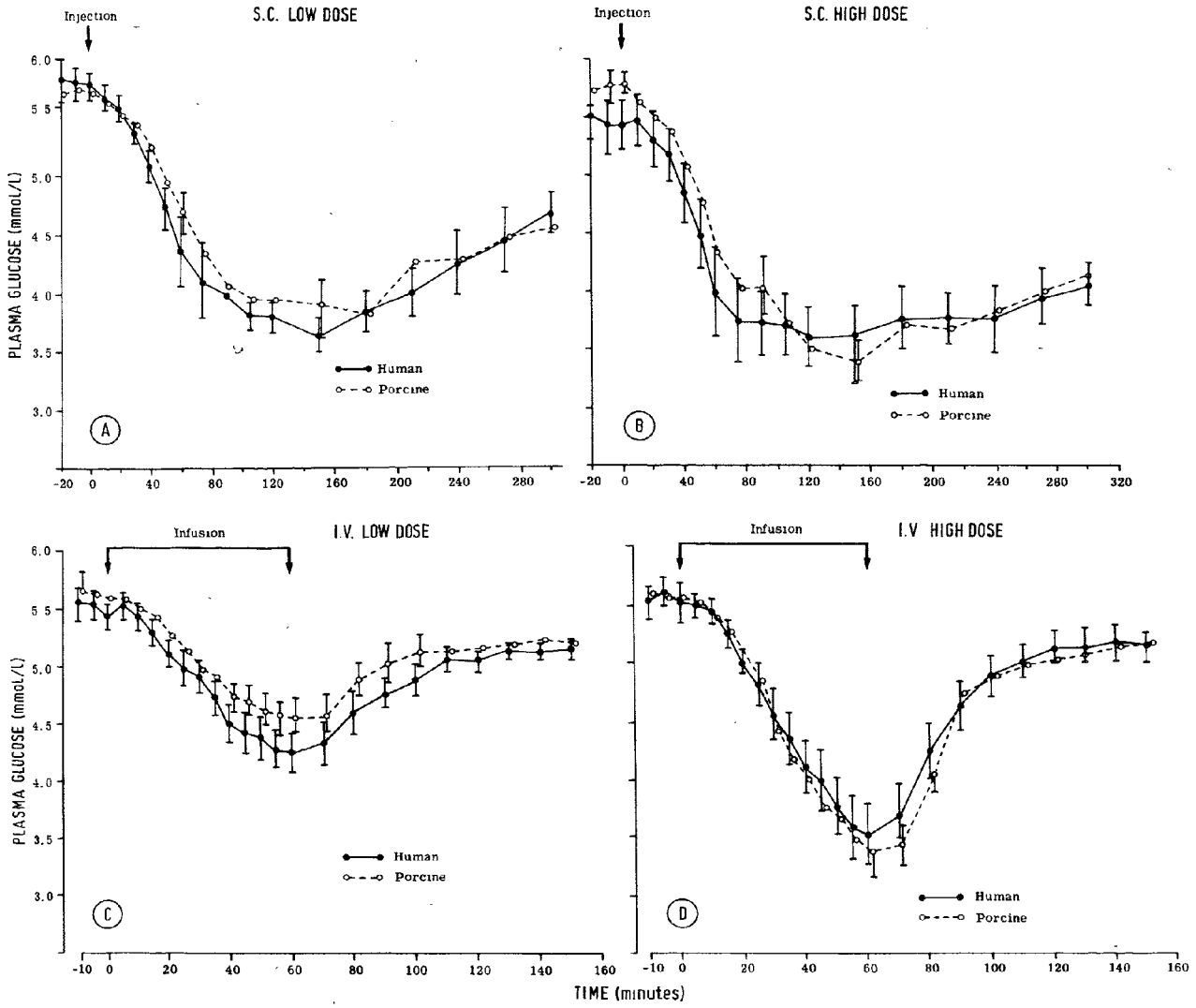
Analysis

For patient security during the experiments, plasma glucose concentrations were measured with a Yellow Springs glucose analyser. The plasma glucose values reported in this paper were estimated in duplicate by a glucose oxidase method using an 'Auto-Analyzer AA2' (Technicon) within 24 h. Blood was also collected for biochemical and immunological analyses (to be reported later). Statistical significance of differences was assessed by paired t tests. Slopes were calculated by the method of least squares.

TABLE I—LOCAL SKIN REACTIONS TO INTRACUTANEOUS INJECTIONS OF HUMAN AND PORCINE INSULIN, DILUENT, AND SALINE CONTROLS

Time after Injection	Insulins						Controls					
	Porcine			Human			Diluent			Saline		
	E	S	B	E	S	B	E	S	B	E	S	B
5 min	4	0	0	3	0	0	1	0	0	1	0	0
30 min	8	10	0	8	9	0	4	2	0	1	1	0
5 h	5	3	0	6	2	0	1	2	0	0	0	0
24 h	0	0	7	0	0	3	0	0	2	0	0	0
48 h	0	0	6	0	0	2	0	0	1	0	0	0

The pooled number of measurable skin responses at each of the four insulin concentrations and for the single insulin diluent and saline control sites are shown. (E= erythema; S= swelling; B= bruise). There was no trend of response with insulin concentration. No lesion exceeded 0·4 cm in diameter.



Responses to human and porcine insulins, administered subcutaneously and intravenously, at low and high dose.

Mean values (mmol/l) and standard errors for the glycæmic responses to human and porcine insulins injected subcutaneously at low dose (a) and high dose (b); and to the insulins infused intravenously for 1 h at low rate (c) and high rate (d). The time scales differ in the two experiments. Insulin was injected subcutaneously (a, b) or intravenous infusion started (c, d) at zero time. The infusion was stopped at 60 min. Each point plotted represents a sampling time.

RESULTS

A. *Skin Sensitivity Tests*

The local responses to intracutaneous injection of the two types of insulin at four dose levels, together with diluent and saline controls are summarised in table I. Local reactions were minor and consisted of small areas of erythema, swelling, and bruising at the injection sites. Both insulins and the diluent gave more small reactions at the injection site than the saline control but did not differ among themselves in frequency, type, or severity.

B. *Subcutaneous Insulin Injections*

The mean (± SEM) plasma glucose responses to high and low doses of human and porcine insulin injected subcutaneously are presented in the figure, a, b. The mean baseline plasma glucose values before the low dose (4·8 units porcine or human equivalent) insulin injection were very similar as were the values during the period of plasma glucose decline. However, the mean decrement of plasma glucose after human insulin exceeded that after porcine insulin at all time points (table II). In the comparison of glycæmic response to high-dose insulin (9·6 units porcine or human equivalent), that relationship is reversed. Although basal glycæmia was slightly higher before porcine insulin, the absolute decrements after injection were greater than those after human insulin, significantly so at 150, 180, 210, and 300 min (table II).

TABLE II—MEAN DECREMENTS OF PLASMA GLUCOSE FOR THE LATTER 150 MIN OF THE OBSERVATION PERIOD FOLLOWING SUBCUTANEOUS INJECTIONS OF THE TWO INSULINS AT LOW AND HIGH DOSE

—	150 min	180 min	210 min	240 min	270 min	300 min
Low dose:						
Human	−2·16	−1·96	−1·81	−1·54	−1·36	−1·12
Porcine	−1·81*	−1·89	−1·45	−1·46	−1·26	−1·18
High dose:						
Human	−1·82	−1·69	−1·68	−1·69	−1·50	−1·40
Porcine	−2·36†	−2·04†	−2·08†	−1·92*	−1·78	−1·63†

*= $p<0·1$; †= $p<0·05$.

C. *Intravenous Insulin Infusions*

Plasma glucose concentration fell faster and achieved lower nadir values with high concentration insulin infusions (1·7 units/h or human equivalent) than with low (1·0 units/h or equivalent) whether porcine or human (figure, c, d). As with the subcutaneous injection experiments, the hypoglycæmic response to low concentration infusion of human insulin slightly exceeded that with porcine insulin, while at high concentration this relationship was reversed. These trends, though consistent, were small and differences between human and porcine responses were not statistically significant at any single time point. The rate of decline of plasma glucose, calculated for each subject as the slope of the straight line of best fit for the linear portion of the concentration fall during the infusion (table III), was clearly greater at high than low concentration infusions but showed no consistent difference between human and porcine insulins at either concentration.

DISCUSSION

The rapid advance of recombinant DNA technology over the past few years has had as a major goal the production of therapeutically valuable peptides. Human insulin has been high among these. Existing methods of insulin production involve, and are to some extent limited by, the availability of animal pancreas; there may be advantages in the use of the human hormone in man. The experiments described in this paper report not only the first use of human insulin produced by recombinant DNA but also, to our knowledge, the first use of any recombinant DNA product in man. The still evolving science of recombinant DNA technology, especially in respect of insulin production, is well reviewed by Miller and Baxter.[5] Our purpose was to confirm the safety and the efficacy of this product in healthy volunteers.

Injection of a wide range of doses intracutaneously gave no hint of abnormal skin reaction to the product over an inspection period up to 48 h; such trivial reactions as were seen were also shown by the purified porcine insulin solution and by the insulin diluent control. In neither this nor our subsequent experiments were there any indications of short-term adverse effects. Longer-term studies, particularly in respect of immune responses, are clearly of major importance and are in progress.

As to efficacy, to the extent that it can be judged from the comparisons of blood glucose depressing activity in normal subjects, when administered both by the subcutaneous and intravenous routes (summarised in the figure a-d), there seems to be little demonstrable differ-

TABLE III—RATES OF DECLINE OF PLASMA GLUCOSE*

	Low dose		High dose	
Subject	Human	Porcine	Human	Porcine
1	−0·019 (0·003)	−0·018 (0·005)	−0·026 (0·002)	−0·022 (0·002)
2	−0·035 (0·003)	−0·035 (0·003)	−0·040 (0·003)	−0·036 (0·003)
3	−0·033 (0·001)	−0·039 (0·003)	−0·049 (0·001)	−0·064 (0·004)
4	−0·022 (0·004)	−0·023 (0·004)	−0·053 (0·002)	−0·049 (0·001)
5	−0·043 (0·005)	−0·027 (0·002)	−0·035 (0·003)	−0·055 (0·002)
6	−0·025 (0·006)	−0·023 (0·002)	−0·063 (0·004)	−0·062 (0·004)
Mean	−0·030	−0·028	−0·044	−0·048

* Decline in plasma glucose (mmol l^{-1} min^{-1}) with standard errors calculated for each subject from plasma glucose concentrations between 15 min after start of infusion and, for low dose, 40 min after start of infusion, and, for high dose, 10 min before glycæmic nadir.

ence between the purified porcine and the genetically synthesised human insulins. In the subcutaneous experiments the degree of glucose depression produced by the two hormone preparations was very similar at each of the two dose levels selected. In both, the effect of increasing the dose was to prolong the duration rather than to increase the degree of hypoglycæmia. At the higher dose, the mean absolute fall of plasma glucose concentration was statistically significantly greater after porcine than after human insulin for the latter half of the 5 h observation period. At the lower dose the relationship of the hypoglycæmic potencies reversed—hinting perhaps at different slopes of the dose-response curves for the two insulins in man.

A similar pattern suggesting slightly greater mean hypoglycæmic potency for the human insulin at the lower dose infusion rates and for porcine insulin at the higher dose infusion rates was seen in the intravenous experiments. However, this relationship was not consistent for each subject when paired responses were individually compared, and the difference did not reach statistical significance. The rate of glycæmic recovery after the infusion was stopped at 1 h was similar for the two insulin preparations at both doses. We were struck by the consistency of the pattern of hypoglycæmic response and recovery seen within individuals at the same insulin dose, whether porcine or human, and will return to this when the additional data collected in these experiments are available.

The concentrations of insulin, hence infusion rates, were selected mainly on the basis of the experiments of Sacca et al.[6] Their low and high infusion rates, calculated pro rata for body-weight, would have been 1·05 and 1·68 units/h for a 70 kg person. Since the comparisons we wished to make were between insulins within individuals we chose 1·0 and 1·7 units/h as low and high rates, respectively. Sacca et al. observed the effects of the two infusion rates on glucose concentration and changes in specific activity occurring in healthy subjects receiving a continuous infusion of tritiated glucose. They concluded that the fall in plasma glucose concentration at the low insulin infusion rate was virtually entirely attributable to inhibition of hepatic glucose release. At the high rate the fall in glucose concentration was due both to hepatic inhibition and to stimulation of peripheral glucose uptake. Our finding that human insulin might be more potent than porcine at low dose but not at high dose suggests that it may have more effect on the liver and less at the periphery. This may be due to a higher hepatic extraction rate of human than of porcine insulin by the normal liver. The results of the venous plasma insulin assays and C-peptide concentrations in our experiments may cast further light on this speculative interpretation of our findings.

We have, therefore, succeeded in demonstrating, in a small number of intensively studied healthy volunteers, the safety and efficacy of a human insulin preparation produced in *E. coli* by recombinant DNA technology. This is but the first step in a longer process of investigation of its actions, validation of clinical efficacy in diabetics, and continuing vigilant surveillance for unexpected adverse effects.

We are grateful to the seventeen volunteers, recruited from the Department of Medicine, Guy's Hospital, and the Lilly Research Centre, who unfailingly presented themselves for the experiments; to Ms Andrea Collins, Mr David Mackintosh, Mr Andrew Salter, Mr Talat Omer, Dr Martin Mattock, and Mr Richard Rogers; also to Mrs Jean Noble, our unstinting nursing assistant, Ms Joan Caldwell who performed the statistical analyses, and our secretarial assistants.

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CYTORECEPTOR ASSAY FOR 1,25-DIHYDROXYVITAMIN D_3: A NOVEL RADIOMETRIC METHOD BASED ON BINDING OF THE HORMONE TO INTRACELLULAR RECEPTORS IN VITRO

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Summary A new assay for 1,25-dihydroxyvitamin D_3 in biological fluids is described, in which measurement is based on the translocation of the hormone across cell membranes and its binding to specific cytoplasmic receptors inside target cells in culture. The technique obviates the need for chromatographic separation of $1,25(OH)_2D_3$ from other metabolites of vitamin D_3.

INTRODUCTION

THE recognition of the role of 1,25-dihydroxyvitamin D_3($1,25[OH]_2D_3$), the hormonal form of vitamin D_3, in normal and disordered calcium metabolism has resulted in an increasing demand for its measurement in biological fluids.[1-3] The most widely used methods for this measurement are competitive binding radioassays which utilise as binding proteins either preparations of the natural cytoplasmic receptor for $1,25(OH)_2D_3$ or antibodies raised against the hormone.[4-7] However, the available $1,25(OH)_2D_3$ assays are laborious because of the necessity for chromatographic separation of $1,25(OH)_2D_3$ from other metabolites of vitamin D_3. We here describe a novel approach to the measurement of $1,25(OH)_2D_3$ in biological fluids which may obviate some of the technical difficulties of other assay procedures.

The principle behind our method is based on the concept that $1,25(OH)_2D_3$, like classical steroid hormones, diffuses freely across cell membranes and is retained inside its target cells. This "internalisation" occurs because of the high affinity and specific binding of $1,25(OH)_2D_3$ to the intracellular receptor.[1] To exploit this concept we used