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Matched Glucose Responses to Insulin Administered Subcutaneously and Intravenously

Evidence for Subcutaneous Inactivation of Insulin

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Summary. A new technique of programmed intravenous insulin infusion at a series of decreasing rates has been used to imitate the magnitude and time course of biological responses obtained by the subcutaneous route. Groups of normal rats prepared with indwelling venous cannulae were injected subcutaneously with soluble porcine insulin, 0.4 U/kg. The pattern of the resulting hypoglycaemic response was subsequently matched by a 2-hour intravenous insulin infusion at rates decreasing stepwise from 0.3 to 0.05 U $kg^{-1}h^{-1}$. The total amount of insulin infused intravenously was only 50% of that required subcutaneously. In addition, subcutaneous or intravenous infusions of insulin at 0.05 U $kg^{-1}h^{-1}$ were given to two groups of rats from the same batch. When both infusions were continued until plateau responses were reached, a significantly greater lowering of plasma glucose was caused by the intravenous route. These results suggest that when insulin is given subcutaneously significant inactivation of the insulin occurs at or near the injection site.

Key words: Insulin injection, insulin infusion, subcutaneous, intravenous, plasma immunoreactive insulin, plasma glucose measurement, rat.

Most peptide hormones, including insulin, are administered subcutaneously, and are believed to be subject to the action of a wide range of tissue proteases. We have reported elsewhere evidence obtained in the chick that two unrelated peptides (parathyroid hormone and calcitonin) are partially inactivated at the injection site and can be protected by addition of protease inhibitors [1]. Local monitoring of the injection site after giving isotopically labelled insulin indicates that radioactivity disappears rapidly [2–8], but it cannot be assumed that the labelling atoms remain within bioactive molecules. Data obtained by radioimmunoassay also requires critical evaluation because of the lack of correlation between immunological and biological activity in many peptide fragments, and few bioassays are sufficiently sensitive to follow blood levels. The present paper illustrates a new approach, based on the comparison of subcutaneous and intravenous infusion, by which pharmacokinetic information can be obtained without the use of isotopically labelled hormones.

Methods

Animals

Male Wistar rats, 350–400 g, were fed on rat and mouse diet No 1 (British Petroleum). They had uninterrupted access to water but food was removed 1 h prior to each experiment.

Cannulation

At least three days before the first experiment, two indwelling polyvinyl venous cannulae (Code No 800/000/125, 19 gauge i. d., from Portex Limited, Hythe, Kent, and size P1, 27 gauge i. d., from Braun Apparatebau, Melsungen, Germany) were inserted under anaesthesia with pentobarbitone sodium (40 mg/kg, IP). The cannulae, the smaller for infusion and the larger for blood sampling, were tied in the external jugular veins so that their tips lay in the superior vena cava [9]. An infusion cannula was implanted in the subcutaneous tissue of the rat's back [10].

Infusion Apparatus

A harness [11] was fitted to each rat and the exposed end of the infusion cannula threaded through the centre of "curtain-wire" which travels up vertically from the rat's back to a swivel [12] interposed between cannula and infusion pump. This infusion apparatus allows the rats relatively unrestricted movement and

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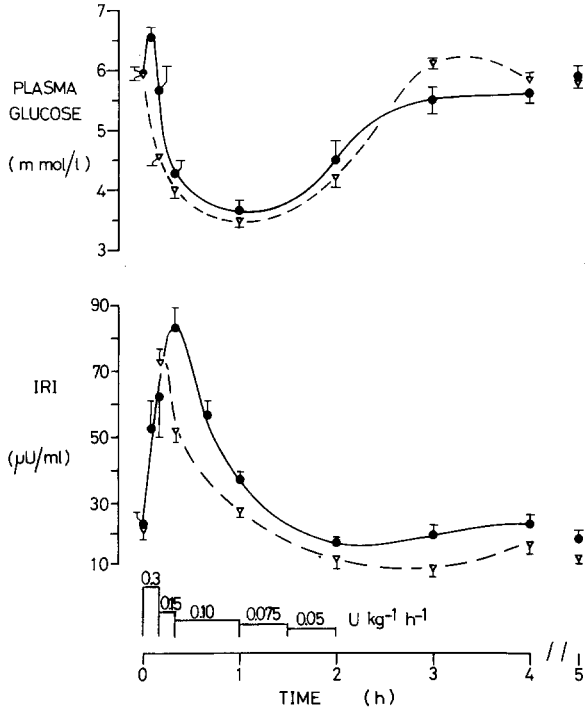

Fig. 1. Comparison of the mean plasma glucose and immunoreactive insulin levels (± SEM) produced by subcutaneous injection of insulin (0.4 U/kg) (●) or intravenous infusion of insulin (0.2 U/kg) (▽) at programmed rates, decreasing stepwise as shown and stopping at 2 hours, to groups of 9 rats

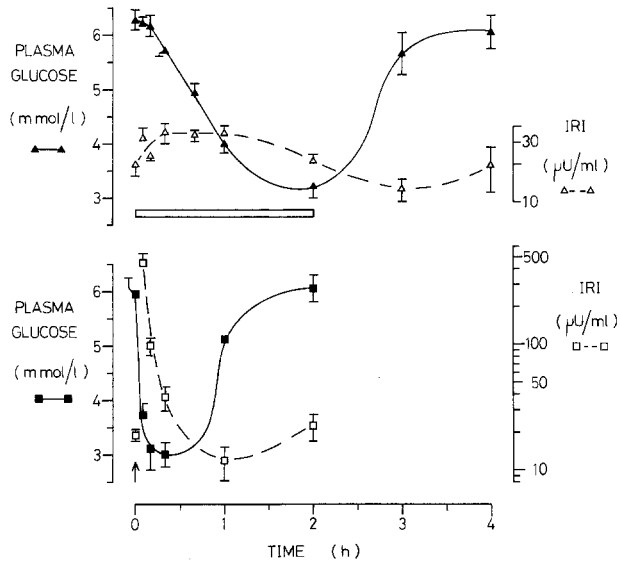

Fig. 2. Mean plasma levels of immunoreactive insulin and glucose produced by administering insulin (0.2 U/kg) as a constant intravenous infusion over 2 hours (□) (upper panel) or as a single intravenous pulse (↑) (lower panel) in two groups of 6 rats (± SEM)

protects the tubing. A Watson-Marlow flow-inducer (MHRE 88L) was connected by 4 to 1 gearing to two multi-channelled peristaltic manifolds so that up to 20 rats could be infused simultaneously at low rates (0.15 to 1.5 ml/h).

Sampling

Blood samples, 0.3 ml, were withdrawn into syringes containing 10 μl EDTA solution (100 mmol/l) [9]. The plasma was analysed for glucose using the Beckman Glucose Analyzer and the remainder frozen for subsequent double-antibody radioimmunoassay of insulin (kit from Radiochemical Centre, Amersham). Standards of porcine insulin were employed and the antiserum used was tested to confirm linear cross-reactivity with rat insulin (Novo Industri, Copenhagen). The sensitivity of the assay was 5 μU/ml and the within assay coefficient of variation was ≤10.5%.

Insulin Administration

Highly purified porcine insulin (Actrapid, Novo Pharmaceuticals) was infused IV in a vehicle containing 2% (v/v) rat serum and sodium acetate trihydrate, 1 g/100 ml. This vehicle was heated for 1 h at 56 °C and subsequently adjusted to pH4 by addition of concentrated hydrochloric acid and sterilised by membrane filtration (Millipore grade HA membranes, average pore size 0.45 μ). No insulin adsorption could be detected when a 2-ml solution of insulin (80 mU/ml) in the vehicle was exposed to polystyrene of surface area 342 cm^2 (method [3]).

Subcutaneous insulin injections of 0.1, 0.2 and 0.4 U/kg (adjusted to pH7) were administered in a constant volume of 0.2 ml to the medial surface of the thigh.

Separate experiments were carried out to ascertain whether an infusion was equally effective SC and IV. A group of 16 rats was randomly divided into two groups and infused at 0.15 ml/h either SC or IV, through tubing of identical lengths, using insulin solutions calculated to deliver 0.05 U/kg/h on one day and 0.1 U/kg/h on another.

Results

SC injection of the appropriate vehicle alone produced a small initial increase in plasma glucose (+ 15% at 10 min). Glucose levels returned to normal by 45 minutes. Intravenous infusion of the vehicle at 0.5 ml/h produced a small continuous fall in glucose, amounting to 9.1 ± 0.1% (± SEM) of the baseline value by the end of a 2-hour infusion. Plasma levels of endogenous insulin showed no significant change with vehicle given SC or IV.

SC injection of insulin produced the small initial increase in glucose described in the control animals but dose-related hypoglycaemia then developed (Table 1). Plasma insulin levels reached a peak 20 min after the injection of 0.4 U/kg and had returned to control levels by 2 h (Fig. 1). This resulted in a mean fall in glucose of 38.3 ± 0.03% (± SEM) at 60 min, with virtually complete recovery by 3 h.

Table 1. Dose-response relationship of insulin injected subcutaneously to rats. (Results are expressed as a percentage of the starting plasma glucose level ± SEM; number of observations)

Insulin (U/kg)	Time (min)			
	5	10	30	60
0	–	115.1±0.02 (5)	–	96.2±0.03 (5)
0.10	–	–	95.4, 96.3	93.5, 95.0
0.15	–	–	87.0, 90.0	93.0, 95.4
0.20	–	–	72.0±6.0 (4)	76.6±7.4 (4)
0.40	109.4±0.03 (4)	91.1±0.05 (4)	65.7±5.0 (4)	62.8±1.2 (4)

When insulin (0.2 U/kg) was administered as a single IV injection, plasma insulin levels reached 450 μU/ml at 5 min while the same dose infused IV over 2 hours produced an insulin concentration of 36 μU/ml resulting in a greater biological response (Fig. 2).

In an attempt to imitate the probable time course of absorption of the SC dose of insulin (0.4 U/kg), half of this total dose was then infused IV at programmed rates, decreasing stepwise from 0.3 to 0.05 U/kg/h over two hours (Fig. 1). This produced a peak of plasma insulin similar to that produced following SC injection, but of slightly quicker onset and shorter duration. The resulting hypoglycaemia very closely imitated the magnitude and time course of the SC response (Fig. 1).

A direct comparison was also made of IV and SC infusions, continued in each case for long enough to obtain a stable degree of hypoglycaemia. The paired infusions were carried out simultaneously in two groups of rats from the same batch. Infusion at 0.05 U/kg/h produced glucose lowering of 3.3 mmol/l by the IV route while SC infusion produced a fall of only 2.2 mmol/l even after 6 h ($p < 0.01$) (Fig. 3). Doubling the infusion rate to 0.1 U/kg/h did not produce plateau blood glucose levels by the end of the 4 h infusion but resulted in a glucose fall of 3.7 mmol/l irrespective of the route of administration, presumably reflecting the critical level at which other hormonal mechanisms are able to maintain blood sugar.

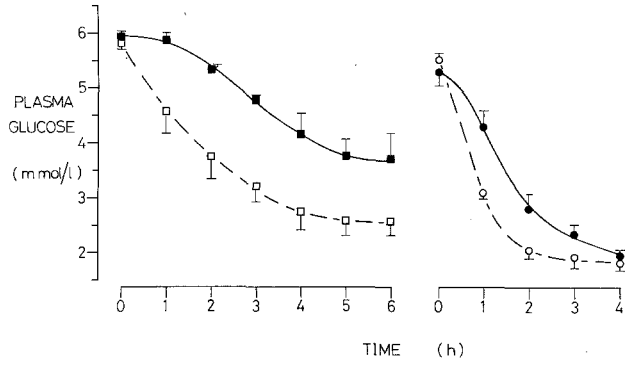

Fig. 3. Mean plasma glucose levels (± SEM) in two groups of 8 rats from the same batch infused with insulin, a) at 0.05 U kg^{-1} h^{-1} either subcutaneously (■) or intravenously (□), (left hand panel), or b) at 0.10 U kg^{-1} h^{-1} either subcutaneously (●) or intravenously (0) (right hand panel). In all cases, insulin infusions commenced at time zero and continued throughout the periods illustrated

Discussion

One indication of substantial inactivation after SC injection of peptides can be found by comparing typical SC and IV dose requirements for bioassays. In the absence of local destruction, the relatively slow absorption from an SC site would minimise drug wastage resulting from concentration-dependent rates of metabolism and excretion and should lead to a *lower* dose requirement by the SC route, exactly the opposite of what is found on comparing SC and IV bioassays of a range of peptide hormones [1].

The absorption of insulin from an SC site has been extensively studied by isotopic or immunoassay methods [2–8, 14, 15] and although the studies with tritiated insulin may reflect bioactive levels the evidence is indirect. The present study in normal rats illustrates a new approach by which pharmacokinetic information can be obtained without the use of isotopically labelled insulin. Since the hypoglycaemic response to SC injection of insulin depends on the magnitude and rate of entry of bioactive insulin to the circulation (see Fig. 2) the response can be imitated by controlled IV infusion only if the concentrations of bioactive insulin reaching the target tissues are very similar. When this was achieved by programmed IV infusion, only half the SC dose was required (Fig. 1). A difference in endogenous insulin secretion or in food absorption associated with the two procedures is possible but seems unlikely since the experiment was designed to produce an identical time course and magnitude of hypoglycaemia by the two routes, and the unchanged levels of endogenous

insulin measured in animals receiving the control infusion confirm that the animals were in a steady-state condition prior to the administration of exogenous insulin. Therefore, the results suggest that up to 50% of the insulin was inactivated at the SC site. This hypothesis was confirmed by other recent work using high specific activity tritiated insulin chemically indistinguishable from the native hormone. A significant portion of labelled porcine insulin was degraded at the SC site following injection to rats [14] and a mean of 21% was degraded following injection to anaesthetised pigs [15]. As in the results reported here SC insulin is absorbed substantially faster in rats than in man and pigs, presumably reflecting the differences in histological structure of the SC tissues between species.

Local degradation might account for the higher dose requirements when insulin is administered SC than IV during attempts to maintain normoglycaemia in diabetic subjects [10, 16, 17]. However, dose requirements by the SC and IV routes can only validly by compared by infusing the insulin in both cases under near-equilibrium conditions, since high insulin levels reduce blood glucose by causing increased uptake in muscle and adipose tissue, whereas low concentrations principally diminish hepatic glucose release [18]. In normal rats (Fig. 3) and in a recent study on normal and diabetic man [19] a significantly greater lowering of plasma glucose was caused by the IV route when insulin was infused at identical rates either SC or IV. It is also clear from other published comparisons of the IV, SC and intramuscular routes of insulin administration in normal subjects [20] and in lean and obese diabetics [21] that the mechanisms and magnitude of hypoglycaemic responses vary according to the rate of entry of insulin to the circulation.

The present paper demonstrates that it is possible to mimic the response to SC insulin by programmed infusion IV to achieve the same pattern of entry of hormone to the circulation and that this simple method can provide information on the kinetics of absorption of *biologically active* material from the SC site.

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