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Originals

Demonstration of a relatively hepatoselective effect of covalent insulin dimers on glucose metabolism in dogs

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Summary Insulin analogues with relatively greater effect on hepatic glucose production than peripheral glucose disposal could offer a more physiological approach to the treatment of diabetes mellitus. The fact that proinsulin exhibits this property to a minor degree may suggest that analogues with increased molecular size may be less able than insulin to obtain access to peripheral receptor sites. Covalent insulin dimers have previously been shown to possess lower hypoglycaemic potencies than predicted by their in vivo receptor binding affinities. Reduced rates of diffusion to peripheral target tissues might be an explanation for the lower in vivo potency compared to insulin. To test the relative hepatic and peripheral effects of covalent insulin dimers, glucose clamp procedures with D-[3-^{3}H]glucose tracer infusions were used in anaesthetised greyhounds to establish dose-response curves for rates of hepatic glucose production and glucose disposal with insulin, $N^{\alpha B1}$, $N^{\alpha B'1}$,-suberoyl-insulin dimer, and $N^{\varepsilon B29}$, $N^{\varepsilon B'29}$,-suberoyl-insulin dimer. With $N^{\alpha B1}$, $N^{\alpha B'1}$,-suberoyl-insulin dimer molar potencies relative to insulin were 68 %, (34–133) (mean and 95 % fiducial limits), for inhibition of hepatic glucose production and 14.7 %, (10.3–20.9) for glucose disposal. With $N^{\varepsilon B29}$,$N^{\varepsilon B'29}$,-suberoyl-insulin dimer potencies were 75 %, (31–184) and 2.5 %, (1.5–4.3), for inhibition of hepatic glucose production and for glucose disposal, respectively. The demonstration that both dimers exhibit a significantly greater effect on glucose production than on glucose disposal supports the suggestion that analogues with increased molecular size may exhibit reduced ability to gain access to peripheral target cells. [Diabetologia (1995) 38: 1007–1013]

Key words Insulin, insulin analogues, glucose metabolism, euglycaemic clamp, insulin action, hepatoselectivity, glucose production.

Insulin influences glucose metabolism in vivo by inhibiting hepatic glucose production (Ra) and by stimulating peripheral glucose disposal (Rd) mostly in skeletal muscle [1]. There have been suggestions that some insulin analogues differ in their potencies relative to insulin for these two separate actions on glucose metabolism [2]. The most well-established quantitative data are derived from extensive studies with human proinsulin. It has been reported that proinsulin, in comparison to insulin, is more effective in the liver than in the periphery [3–5]. The reasons for this are not yet understood.

It is now established that insulin exhibits its biological effects by binding to specific receptors located at the surface of the target cells [6]. The availability of a hormone at the receptor sites in the target tissues is one determinant of its physiological effect. Endothelial cells in peripheral tissues, although less impermeable than the blood-brain barrier, nevertheless

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Abbreviations: B1-B′ 1D, $N^{\alpha B1}$,$N^{\alpha B'1}$,-suberoyl-insulin dimer; B29-B′ 29D, $N^{\varepsilon B29}$,$N^{\varepsilon B'29}$,-suberoyl-insulin dimer; Ra, hepatic glucose production rate; Rd, peripheral glucose disposal rate; M_r, relative molecular weight; MCR, metabolic clearance rate; ANOVA, analysis of variance.

limit the transfer of materials from the circulation into the tissues with the relative rate of diffusion inversely related to molecular size [7]. In contrast hepatocytes are freely in contact with all blood constituents in the hepatic sinusoids. Recently developed insulin analogues which remain monomeric ($M_r \approx 6000$) at concentrations used therapeutically are absorbed substantially more quickly from subcutaneous depot injection sites than conventional preparations of soluble insulin which remain predominantly hexameric under such circumstances [8, 9]. It is therefore clear that molecular size can influence the rate of transfer of insulin from the tissues into the blood. Conversely, the observations that proinsulin ($M_r \approx 9000$) exhibits a relatively greater effect on Ra than RD [4], could be explained if transfer of analogues with increased molecular size out of the circulation into peripheral tissues was similarly reduced in comparison to the rate of transfer of insulin. We have compared the effects of insulin ($M_r \approx 6000$) and two covalently bound insulin dimers (MW 11 604) on Ra, and Rd in anaesthetised greyhounds.

Materials and methods

Materials. B1-B′ 1D and B29-B′ 29D were prepared as previously described [10]. Semi-synthetic human insulin was purchased from Novo Laboratories (Novo Research Institute, Bagsvaerd, Denmark).

Experimental protocol. Euglycaemic glucose clamp procedures [11] each with two rates of infusion of either insulin or one of the two insulin dimers were performed in random order on three intact, adult, overnight fasted anaesthetised greyhounds weighing 28–36 kg. The protocols were approved by the United Kingdom licensing authority for animal experimentation. Each complete study comprised six separate 8-h experiments (protocols 1 and 2) with an interval of at least 4 weeks between the experiments. Anaesthesia was induced by intravenous injection of sodium pentobarbitone (30 mg/kg body weight). The dogs were ventilated at minute volumes previously shown to maintain normal blood oxygen and carbon dioxide concentrations [12]. The body temperature was maintained at 37 °C using a heating pad. Catheters were inserted into a cephalic vein for the infusion of D-glucose and D-[3-^{3}H]glucose, a lateral saphenous vein for infusion of insulin or dimers and an external jugular vein for blood sampling. Following an intravenous priming dose (50 μCi) of d-[3-^{3}H]glucose (Radiochemical Centre, Amersham Ltd, Chalfont, Bucks., UK) a continuous infusion of D-[3-^{3}H]glucose 0.5 μCi/min was initiated. After 120 min for equilibration of glucose tracer and a 30-min period for baseline observation a primed two-step infusion of insulin or dimer was started.

Insulin or dimer for infusion was diluted into 0.9 % (w/v) sodium chloride in water containing 1 % (w/v) human serum albumin (Blood Products Laboratory, Therapeutics Division, Elstree, Herts., UK) to achieve infusion rates of 3.4 and 13.8 pmol · kg^{-1} · min^{-1} (insulin), 5.0 and 24.8 pmol · kg^{-1} · min^{-1} (B1-B′ 1 D and B29-B′ 29 D) in protocol 1 and 6.9 and 68.9 pmol · kg^{-1} · min^{-1} (insulin), 10.0 and 49.5 pmol · kg^{-1} · min^{-1} (B1-B′ 1 D and B29-B′ 29 D) in protocol 2. Priming doses were calculated assuming an apparent distribution space of 100 ml/kg [2] and previously published values for insulin and insulin dimer clearance rates in dogs [12]. After 120 min the infusion rate was increased following a second appropriate priming dose and continued for a further 120 min. Four blood samples were drawn at 10-min intervals for immediate determination of basal (120–150 min) plasma glucose concentration. After the initiation of insulin or dimer infusion (150 min), blood samples were drawn at 5-min intervals to 480 min for serum glucose determination in order to maintain the glucose clamp. An infusion of 50 % (w/v) D-glucose in water was modulated to maintain euglycaemia. Blood samples were also collected every 30 min and at 10-min intervals from 120–150 min (baseline determination), 240–270 min and 360–390 min for determination of immunoreactive insulin and glucose concentrations and the plasma glucose specific activity. Insulin or dimer infusion was discontinued at 390 min. Blood sampling was continued every 5 min to 430 min and at 10-min intervals thereafter for the measurements of plasma glucose specific activity and insulin or dimer concentration. Serum and plasma samples were stored at −20 °C until assayed (within 2 weeks). Insulin and dimer concentrations were determined in serum samples and in the infusion mixtures by double antibody radioimmunoassay [13]. Iodinated human insulin was used as labelled ligand in the radioimmunoassay of insulin and covalently linked insulin dimers. Both dimers and insulin showed similar cross-reactivity with the antibody selected. For estimation of dimer concentrations appropriate in vitro dilutions of each dimer were used as standards within the assay. The within-assay coefficient of variation was 6 %. Plasma and infused glucose concentrations were measured using a glucose oxidase technique on a model 23 AM glucose analyser (Clandon Scientific Ltd, Yellow Springs, Ohio, USA). Within-assay coefficient of variation at 5 mmol/l was 3.5 %. Glucose specific activity was determined in duplicate on deproteinized plasma and tracer infusate according to the method of Somogyi [14], lyophilised, reconstituted in distilled water then counted in Pico Fluor 15 scintillant on a liquid scintillation counter (1219 Rackbeta; LKB, Turku, Finland).

Calculations. Values for total and endogenous Ra at all time points were calculated by the method of Mari [15] in which the glucose concentration and specific activity data are smoothed by a modified version of the optimal segments programme [16]. This output is applied to a two compartment model of glucose distribution and metabolism. As in this experimental protocol euglycaemia was maintained constant by a variable infusion of exogenous glucose, total Ra has been taken to represent Rd. Values of the metabolic clearance rate (MCR) of the hormones were calculated from their concentrations during the last 30 min of each infusion period. No correction was made for persisting endogenous insulin secretion which in these studies was not actively suppressed.

MCR (ml · kg^{-1} · min^{-1}) = infusion rate (pmol · kg^{-1} · min^{-1}) ÷serum hormone concentration (pmol/ml).

MCR of glucose was calculated from the glucose concentration during the last 30 min of each infusion period.

MCR (ml · kg^{-1} · min^{-1}) = glucose Rd (μmol · kg^{-1} · min^{-1}) ÷plasma concentration (mmol/l).

Statistical analysis

The last 30 min of each infusion period was considered for statistical analysis. For comparison between groups two-way analysis of variance (ANOVA) was used and the significance of differences between the means tested by Fisher's least squares

test using Number Cruncher Statistical System software (Hintz, Kaysville, Utah, USA). The results are expressed as arithmetic mean ± SEM and p-values less than 0.05 were considered statistically significant. Potency estimates for each dimer compared with insulin were obtained from the linear portion of the log-dose response curves using the parallel line bioassay technique described by Finney [17]. Results are expressed on a molar basis, i.e.: Only one of the two insulin moieties in a dimer would need to be fully active to produce a calculated potency of 100 %.

Results

Serum insulin and dimer concentrations

Protocol 1. Serum insulin, B1-B′ 1 D and B29-B′ 29 D concentrations are shown in Figure 1. Following infusion of insulin at 3.4 and 13.8 pmol · kg^{-1} min^{-1}, insulin concentrations were 0.21 ± 0.02 (240–270 min) and 1.41 ± 0.29 nmol/l (360–390 min), respectively. These insulin concentrations were significantly different from basal levels (0.07 ± 0.01 nmol/l) $p < 0.01$. Apparent basal insulin concentrations in the studies with dimers, i.e.: assayed against dimer standards were all less than 0.06 nmol/l. Following infusion of dimers at 5.0 and 24.8 pmol · kg^{-1}. min^{-1}, concentrations of B1-B′ 1 D were 0.60 ± 0.58 and 4.87 ± 0.30 nmol/l and B29-B′ 29 D were 1.03 ± 0.10 and 5.51 ± 0.39 nmol/l, respectively.

Protocol 2. Following infusion of insulin at 6.9 and 68.9 pmol · kg^{-1} · min^{-1}, insulin concentrations were 0.55 ± 0.03 and 16.22 ± 2.7 nmol/l. Following infusion of dimers at 10.0 and 49.5 pmol · kg^{-1} · min^{-1}, concentrations of B1-B′ 1 D were 1.34 ± 0.12 and 11.01 ± 0.86 nmol/l and B29-B′ 29 D were 2.08 ± 0.38 and 11.89 ± 1.12 nmol/l, respectively.

Metabolic clearance rates of insulin and dimers

The MCR of dimers were lower than those of insulin as seen in Figure 2. Rates were 20.3 ± 2.49, 8.88 ± 0.88 and 6.66 ± 0.70 ml · kg^{-1} · min^{-1} for insulin, B1-B′ 1 D and B29-B′ 29 D, respectively, at the lowest infusion rate, falling to 6.07 ± 0.66, 5.25 ± 0.28 and 5.51 ± 0.25 ml · kg^{-1} · min^{-1} at the highest infusion rate (Fig. 2). The decrease in MCR with increasing insulin and B1-B′ 1 D concentrations was significant ($p < 0.05$).

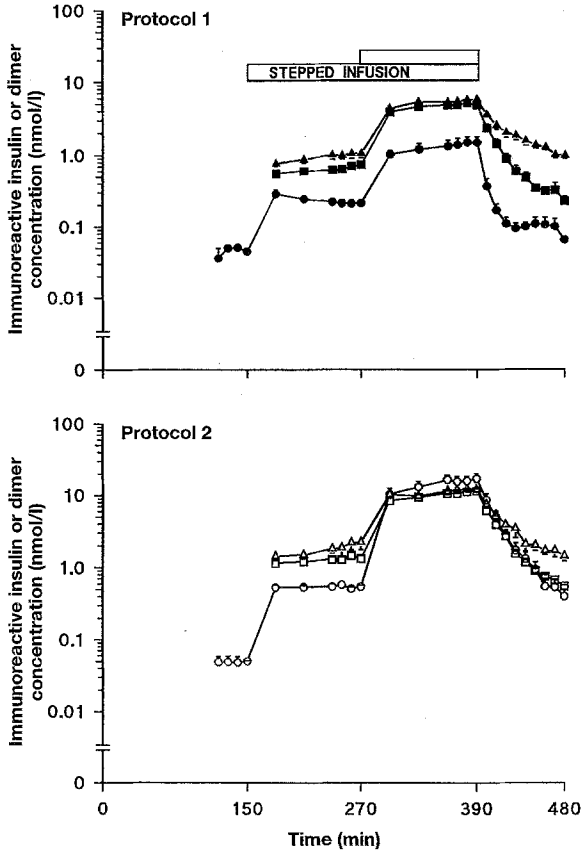

Fig. 1. Immunoreactive serum insulin (●) (○), B1-B′ 1 D (■) (□) and B29-B′ 29 D (▲) (△) concentrations during protocol 1 (closed symbols) and protocol 2 (open symbols) in dogs. Values are mean ± SEM ($n = 3$)

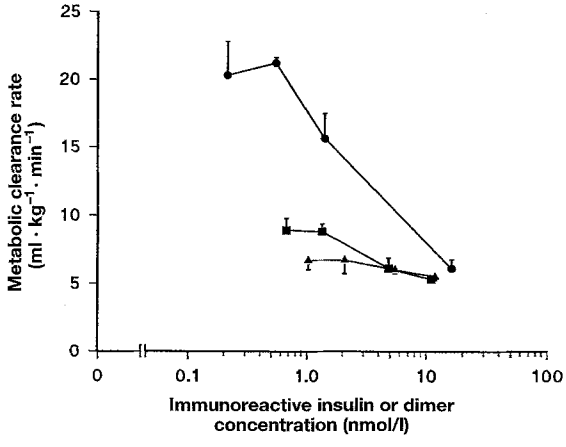

Fig. 2. Relationship between serum concentrations of insulin (●), B1-B′ 1 D (■) and B29-B′ 29 D (▲) and MCR, in protocol 1 and 2. Values are mean ± SEM ($n = 3$)

Glucose metabolism

Euglycaemic glucose clamp. Plasma glucose concentration was 4.8 ± 0.03 mmol/l in the basal state and was maintained between 3.8 and 5.6 mmol/l during the studies, i.e.: at no time point in any experiment was plasma glucose sufficiently raised to stimulate endogenous insulin secretion or low enough to initiate counter regulatory responses.

Hepatic glucose production rate, glucose disappearance rate and glucose metabolic clearance rate. Infu-

sion of insulin and the dimers suppressed glucose Ra and increased glucose Rd, $p < 0.05$, (Fig. 3). Glucose Ra and MCR achieved with the infusion of insulin and the dimers reached an acceptable steady state in the last 30 min of each infusion period (240–270 min, 360–390 min), Table 1. Insulin infusion at the highest rate employed (68.9 pmol · kg^{-1} · min^{-1}), suppressed glucose Ra significantly from a basal value of 11.9 ± 0.9 to −7.7 ± 0.9 μmol · kg^{-1} · min^{-1}. Glucose Rd increased from 11.9 ± 0.9 to 41.2 ± 1.9 μmol · kg^{-1} · min^{-1}, ($p < 0.05$). Infusion of 49.5 pmol · kg^{-1} · min^{-1} of B1-B′ 1 D suppressed glucose Ra to −7.6 ± 1.5 μmol · kg^{-1} · min^{-1} ($p < 0.05$) and increased glucose Rd to 44.9 ± 2.6 μmol · kg^{-1} · min^{-1}, whereas with B29-B′ 29 D, glucose Ra was suppressed to −4.4 ± 1.1 μmol · kg^{-1} · min^{-1} ($p < 0.05$) and glucose Rd increased to 30.6 ± 3.3 μmol · kg^{-1} · min^{-1}. Dose response curves were constructed for glucose Ra and Rd (Fig. 3). Dose response curves were not significantly non-parallel. Molar potencies of B1-B′1 D and B29-B′ 29 D relative to insulin are shown in Table 2.

Both dimers exhibited capacities to reduce glucose Ra which were not significantly different from that of insulin itself but displayed significantly reduced abilities to stimulate Rd.

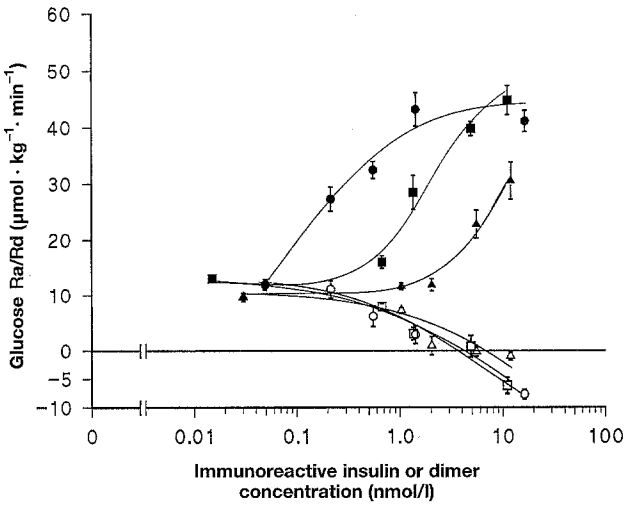

Fig. 3. Dose response relationships of glucose Rd (closed symbols) and glucose Ra (open symbols) to serum concentrations of insulin (●) (○), B1-B′ 1 D (■) (□) and B29-B′ 29 D (▲)(△). Values are mean ± SEM of the last 30 min of the infusions

Discussion

This study was undertaken to examine the differential effects of human insulin and two covalently linked insulin dimers B1-B′ 1 D and B29-B′ 29 D on the rates of glucose Ra and glucose Rd using a hyperinsulinaemic euglycaemic clamp technique. The results reported here demonstrate that the rates of glucose Rd following infusion of the dimers were significantly lower relative to insulin while the rates of glucose production by the liver were similar, despite the fact that higher infusion rates of the dimers were used.

These dimers also exhibited slower rates of metabolism compared to insulin, in agreement with earlier studies using lower infusion rates and during hypoglycaemia [12]. The effect of the persisting endogenous insulin secretion on these values can be considered although not accurately quantitated. Comparison of the basal concentration with those after infusion suggests that this source of error may well have affected the value for insulin MCR at the lowest rate of infusion (Figure 2). Differences between B1-B′ 1 D and B29-B′ 29 D are of interest.It is clear from Figure 1 that although they exibit similar steady-state levels at the end of the infusion periods the decay curves are different. The most likely explanation is that there are differences of distribution even between the two dimers. The fact that the decay curve is not linear on a semi-logarithmic plot as in Figure 1 implies that two or more compartments are involved. The observations are therefore compatible with our suggestion that the dimers differ from insulin (and from each other) in their rates of diffusion across the barriers between tissue compartments.

There are considerable methodological problems associated with accurate measurement of glucose Ra. Under non-steady-state conditions absolute values may be subject to error due to lack of adequately rapid mixing between unlabelled and labelled pools in different physiological compartments [18, 19]. One common manifestation of these problems of equilibration is the generation of apparently negative values for glucose Ra at high insulin concentrations. The use as here of two compartment models has partly solved this problem but as can be seen in Figure 3 the highest concentrations of insulin and of B1-B′ 1 D have still led to apparently negative Ra values. In these experiments no effort was made to maintain glucose specific activity by variable rates of glucose tracer infusion. Such measures can reduce although not abolish equilibration errors in the estimation of glucose turnover [18]. It could be argued that differences in this error could lead to an erroneous apparent hepatoselectivity. However, the magnitude of the error in estimation of Ra will decrease as Rd decreases. The greater the Rd value the greater will be the underestimation of Ra. With two analogues of different biopotencies but equal relative abilities to influence Ra and Rd the error under discussion could lead to the conclusion that the more effective analogue would be relatively hepatoselective. The potential problem therefore adds confidence to our finding that the less potent dimers are in fact the materials which display such an effect.

Insulin analogues with modification of the B1 position have been shown to retain their biological prop-

Table 1. Glucose Ra ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) and glucose MCR ($ml \cdot kg^{-1} \cdot min^{-1}$) at 90, 100, 110 and 120 min after initiation of each infusion rate, i.e.: 240–270 min and 360–390 min

	Insulin		B1-B'1 D		B29-B'29 D	
	Glucose Ra	MCR	Glucose Ra	MCR	Glucose Ra	MCR
Protocol 1						
Time (min)						
a 240	10.83 ± 4.12	6.07 ± 1.23	6.90 ± 1.38[a]	3.50 ± 0.57	6.46 ± 1.27[a]	1.35 ± 0.25[a,b]
b 250	10.43 ± 3.14	6.19 ± 1.36	7.16 ± 1.08[a]	3.69 ± 0.63	6.50 ± 1.19[a]	1.40 ± 0.22[a,b]
c 260	10.93 ± 3.39	6.04 ± 1.31	7.90 ± 0.56[a]	3.93 ± 0.65	7.20 ± 1.10[a]	1.59 ± 0.24[a]
d 270	12.03 ± 3.79	6.10 ± 1.29	9.86 ± 0.16	4.47 ± 0.75	8.76 ± 0.97	1.95 ± 0.23
a 360	1.76 ± 2.83	11.00 ± 2.60	1.73 ± 3.88	9.90 ± 1.02	−0.33 ± 1.27	4.70 ± 1.42
b 370	2.66 ± 3.81	11.25 ± 2.36	1.46 ± 4.64	9.84 ± 0.98	−1.10 ± 2.21	4.93 ± 1.35
c 380	2.70 ± 4.12	11.06 ± 2.63	0.46 ± 4.86	9.53 ± 1.13	0.26 ± 2.07	4.97 ± 1.23
d 390	1.53 ± 2.90	10.72 ± 2.71	−0.46 ± 4.53	9.05 ± 1.14	0.60 ± 2.25	5.05 ± 1.15
Protocol 2						
a 240	5.93 ± 4.85	6.67 ± 1.07[a,b]	2.83 ± 1.30	6.15 ± 2.06	1.46 ± 2.55	6.19 ± 1.95
b 250	5.76 ± 4.53	7.00 ± 0.83[a,b]	2.00 ± 2.53	5.97 ± 1.32	0.30 ± 3.67	5.70 ± 1.40
c 260	6.26 ± 3.67	8.03 ± 1.05	3.06 ± 2.57	6.35 ± 0.98	0.50 ± 4.01	5.99 ± 1.27
d 270	6.93 ± 4.26	8.60 ± 1.16	4.33 ± 3.39	6.78 ± 0.55	1.26 ± 4.58	6.32 ± 0.93
a 360	−7.06 ± 1.56	8.89 ± 1.01	−5.03 ± 4.45	10.39 ± 1.09	−0.07 ± 1.26	10.07 ± 1.78
b 370	−7.70 ± 2.01	8.71 ± 0.72	−5.26 ± 3.38	10.76 ± 0.87	0.60 ± 1.55	10.51 ± 1.17
c 380	−7.73 ± 2.16	8.75 ± 0.60	−6.43 ± 2.61	10.84 ± 1.05	−1.60 ± 1.40	10.55 ± 0.63
d 390	−8.26 ± 2.10	8.44 ± 0.62	−7.93 ± 2.33	10.66 ± 1.14	−2.87 ± 1.20	10.26 ± 0.58

Values are mean ± SEM. [a] Significantly different from d, $p < 0.05$; [b] Significantly different from c, $p < 0.05$

Table 2. Molar potencies with 95 % fiducial limits of B1-B'1 D and B29-B'29 D relative to insulin

	Glucose Ra	Glucose Rd
Insulin	100 %	100 %
B1-B'1 D	68 % (34–133)	14.7 % (10.3–20.9)
B29-B'29 D	75 % (31–184)	2.5 % (1.5–4.3)

erties in vivo and in vitro. Insulin tracers labelled with ^{125}I or ^{3}H on B1 have been shown to behave like native insulin and to display similar MCR values [20, 21]. Studies with insulin analogues which differ from insulin at the B29 position in dogs have shown that these modifications have little effect on the biological activity or clearance of the hormone [2]. It seems likely, therefore, that the differential potencies reported here relate to the dimeric nature of the analogues rather than to an intrinsic effect of either a B1 or B29 modification.

One possible explanation for our findings on Ra (effect on the liver) and Rd (effects on the periphery) relates to the recent suggestion that the insulin receptor structure is to some extent tissue specific [22]. The receptor-dimer interaction may vary in different tissues, though when dimer binding affinities were compared in vitro in isolated liver plasma membranes and adipocytes, they were not different [23]. Although there is no published evidence in dogs, tissue heterogeneity in the structure of the insulin receptor has been reported in the rat [24] and human [25].

Another possible explanation for the differential effect on glucose Ra and Rd could be lower accessibility of the insulin dimers to the peripheral insulin receptors compared to the liver receptors. Such lower accessibility could result from a receptor-mediated transport process through vascular endothelium [26] or differences relating to molecular sieving through the capillary wall and the interstitial space [7, 27]. In vitro studies on the kinetics of lipogenic activity of insulin in fat cells in comparison to fat pad and perfused fat pad suggested limitation of access of insulin to its receptor in perfused fat pad preparations [28]. This can be compared to our animal model where insulin or the dimers of insulin were delivered into a peripheral vein. In order to induce their biological effect in the periphery they have to traverse the endothelial barrier and interstitial fluid to reach the receptors. In the liver the microanatomy is completely different, with no barrier between the liver sinusoids and the surface of hepatocytes. Easy access of insulin and insulin analogues to the binding sites in the liver has been clearly demonstrated in rats [29].

Peripheral administration of proinsulin has been shown to exert a relatively greater effect on Ra than Rd [3, 5], when compared with insulin although the differential was less than we have shown here with these insulin dimers. These observations are compatible with a mechanism dependent on molecular size as proinsulin is intermediate in molecular weight between insulin and insulin dimers.

In studies in dogs where the concentration of insulin in the lymphatic fluid was compared to plasma insulin, concentrations in lymph were lower than those in plasma and correlated better with Rd [30]. Recent

data from human subjects using microdialysis has clearly established the existence of a barrier to transport of insulin from the vascular to the interstitial space [31]. We suggest that this endothelial barrier which exists in the periphery but not in the liver plays an important physiological role in determining access of peptides to receptor sites on muscle cells and other peripheral tissues.

It is of interest that analogues of insulin which remain monomeric ($M_r \approx 6000$) when administered subcutaneously [9, 32], have been shown to appear in the plasma from the subcutaneous depots faster than conventional insulins which are largely hexameric ($M_r \approx 36000$) in the formulations in which they are injected.

In infusion studies in man where the analogues were delivered at high dilution into a peripheral vein, under circumstances in which insulin itself will be largely monomeric glucose Ra and Rd responses were found to be the same for the two monomeric insulin analogues and human insulin suggesting no enhancement of access of these analogues to peripheral sites in comparison to insulin [32].

The results suggest a difference in potency of the two dimers for Rd with respect to insulin. This could reflect differences in accessibility to tissue receptors in spite of the similar molecular mass if in solution the shape adopted by the dimers differed. The possibility that these observations could derive from different capacities of the two dimers to bind with or activate insulin receptors needs to be considered [23]. Analogues with reduced receptor binding potency retain in vivo bioactivity because the increased concentration which results from the lower receptor-mediated MCR compensates for the lower affinity [33]. This effect applies equally to hepatic and peripheral activities as it is related to plasma concentration. Therefore, for a dissociation to be apparent between the hepatic and peripheral potencies a phenomenon additional to such reduced affinity should apply. We suggest that a barrier to peripheral transport provides such a mechanism and that insulin dimers are sufficiently bigger than the monomer for this effect to be apparent.

In conclusion, it appears that the molecular size of an insulin analogue may play an important role in controlling its rate of transfer through capillary endothelial barriers. Insulin-like moieties of high molecular weight, therefore, appear relatively hepatoselective when administered intravenously in vivo. Such hepatoselectivity could allow development of insulin analogues which when peripherally administered for example by subcutaneous injection would more closely reflect the natural balance of activity of native pancreatic insulin for which hepatoselectivity is conferred by virtue of its delivery directly into the portal circulation. Such an analogue could conceivably be monomeric on injection but acquire an increased molecular weight for example by protein binding on reaching the circulation.

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