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ARTICLE

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Tissue selectivity of insulin detemir action in vivo

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Abstract *Aims/hypothesis:* Recombinant DNA technology is a useful tool that can be used to create insulin analogues with modified absorption kinetics to improve glycaemic control in patients with type 1 and type 2 diabetes. Among conventional insulin analogues, which are usually created by amino acid exchange, insulin detemir is the first analogue to be acylated with a fatty acid to enable reversible albumin binding. In this study we determined activation of the insulin receptor (IR)-signalling cascade by insulin detemir at the level of IR and IR substrate (Irs) phosphorylation, as well as downstream signalling elements such as phosphatidylinositol 3-kinase and Akt, and performed epidural EEG in vivo. *Methods:* C57Bl/6 mice were injected i.v. with either insulin detemir or human insulin and Western blot analysis was performed on liver, muscle, hypothalamic and cerebrocortical tissues. Moreover, cerebrocortical activity was detected by EEG in awake mice and cerebral insulin concentrations were measured following human insulin and insulin detemir injection. *Results:* The time course and extent of IR phosphorylation in peripheral tissues were similar following insulin detemir treatment compared with human insulin, but insulin signalling in hypothalamic and cerebrocortical tissue determined by tyrosine-phosphorylation of the IR and Irs2 proteins occurred faster and was enhanced due to a higher insulin detemir concentration in the brain. Moreover, epidural EEG in mice displayed increased cortical activity using insulin detemir. *Conclusions/interpretation:* Taken together, these data suggest that insulin detemir has a tissue-selective action, with a relative preference for brain compared with peripheral tissues.

Keywords Brain · Cerebral cortex · Electroencephalography · Hypothalamus · Insulin analogues · Insulin detemir · Insulin receptor signalling

Abbreviations IR: insulin receptor · Irs: insulin receptor substrate · PI: phosphatidylinositol · p-Tyr: phosphotyrosine

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Introduction

The treatment strategies for patients with type 1 and type 2 diabetes have been continually improved over the past decades. One milestone towards optimised glycaemic control are rapid and long-acting insulin analogues that more closely mimic the physiological insulin response to a meal following injection [1, 2] and achieve sufficient basal day-long glycaemic control [3]. The characteristics are based on the fact that modification of the amino acid sequence of the insulin molecule results in distinguished hexamer formation and therefore altered absorption kinetics. Among conventional insulin analogues that are usually created by amino acid exchange, insulin detemir is the first analogue that is acylated with a fatty acid to enable reversible albumin binding [4–7]. Albumin binding is a common principle to delay absorption and results in retention of the insulin molecule in the s.c. depot for a longer period of time [8]. Due to the profile of action of insulin detemir and the fact that the albumin-bound insulin buffers against rapid changes of absorption, insulin detemir action is supposed to be more predictable in terms of the risk for hypoglycaemic episodes compared with other basal insulins [9–11].

The initiation of insulin therapy in type 1 and type 2 diabetes is usually associated with weight gain [12, 13]. Indeed, there is a positive correlation between improved glycaemic control and weight gain, which is clearly undesirable as obesity is present in most patients with type 2 diabetes.

Over the past years data have accumulated supporting the fact that insulin therapy using insulin detemir leads to both improved glycaemic control and weight stability [14], opening new perspectives for insulin therapy. However, the mechanism(s) underlying the altered weight gain with insulin detemir are unknown.

At the molecular level, insulin binds to its cell surface receptor and stimulates autophosphorylation of the β-subunit, followed by phosphorylation of substrates, including the insulin receptor substrate (Irs) protein family members and activation of the lipid kinase phosphatidylinositol 3-kinase (PI 3-kinase) [15]. In peripheral tissue, activation of this pathway is involved in processes like glucose transport, suppression of hepatic gluconeogenesis, protein synthesis and gene transcription [7, 16]. However, in the brain, intact insulin signalling via the Irs-PI 3-kinase pathway is essential for nutrient homeostasis and appetite regulation as pharmacological inhibition of insulin signalling, especially in the hypothalamus, leads to obesity-induced diabetes [17, 18]. Keeping in mind that insulin detemir therapy is characterised by weight stability or even weight loss, we reasoned that the kinetics of insulin signalling in the brain might be altered by insulin detemir.

In this study we tested this hypothesis and provide in vivo data on the impact of insulin detemir on the insulin-signalling cascade in peripheral and brain tissues. C57Bl/6 mice were injected with either human insulin or the analogue and IR phosphorylation and activation of downstream signalling elements were analysed in muscle and liver and hypothalamic and cerebrocortical tissues. The data suggest that during regular activation of the insulin-signalling cascade in the periphery, insulin detemir displays a predominant activation of the cerebral insulin-signalling cascade.

Materials and methods

Animals Ten-week-old male C57Bl/6 mice were obtained from Charles River WIGA GmbH (Sulzfeld, Germany) and studied after 2 weeks of acclimatisation. All procedures were conducted according to the guidelines of laboratory animal care and were approved by the local governmental commission for animal research. Mice were kept on a normal light/darkness cycle and on a regular chow. Glucose levels were sampled from tail bleeds of ketamine-anaesthetised mice using a Glucometer Elite (Bayer, Elkhart, IN, USA).

In vivo insulin stimulation and Western blot analysis For in vivo stimulation, a bolus of either 1 U/kg body weight of human insulin (0.6 mmol/l, 100 U/ml) or 2 U/kg body weight of insulin detemir (2.4 mmol/l, 100 U/ml) diluted in phosphate buffer was injected into the inferior vena cava of ketamine-anaesthetised mice after an overnight fast. This dose was used to achieve comparable peripheral insulin-signalling kinetics and glucose-lowering effects. Tissues (liver, muscle, hypothalamus and cerebral cortex) were removed at the indicated time points and homogenised at 4°C as described [19]. Homogenates were allowed to solubilise for 30 min on ice and then clarified by centrifugation at 12,000× *g* for 20 min. For detection of insulin-stimulated tyrosine phosphorylation, supernatants were immunoprecipitated with antibodies directed against the carboxy terminus of the IR (KKNGRILTLPRSNPS; a gift from R. Lammers, University of Tübingen, Germany) and Irs2 (No. 06-506; Upstate, Charlottesville, VA, USA). Visualisation of immunocomplexes after gel electrophoresis and Western blotting with anti-phosphotyrosine (*p*-Tyr) antibody (PY20; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-phospho-Akt (Ser473) and anti-Akt (Upstate) was performed with a non-radioactively enhanced chemiluminescence system.

Assay of PI 3-kinase activity The same amount of total protein from tissue lysates was immunopurified with anti-*p*-Tyr antibody and immunocomplexes were absorbed by Protein A-Sepharose for 12 h. Immunoprecipitates were washed three times and pellets were directly incubated with 0.1 mg/ml L-α-phosphatidylinositol (Sigma, Munich, Germany) and 50 μmol/l [γ^{32}P]ATP (Perkin Elmer Life Science, Zaventem, Belgium) at room temperature for 10 min. After addition of 150 μl 1 mol/l HCl, lipids were extracted twice with 450 μl chloroform/methanol (1:1 by volume). Products were separated by thin layer chromatography as described [20]. ^{32}P-labelled phospholipids were detected by autoradiography.

Insulin extracts of total brain Mice were stimulated i.v. with either human insulin (1 U/kg mouse) or insulin detemir (2 U/kg mouse). At the indicated time points, total brain was homogenised in 1.0 ml acid-ethanol and insulin was extracted overnight at −20°C. The following day, samples were centrifuged and insulin concentration was measured in the supernatant with a competitive RIA kit (Linco Research, St Charles, MO, USA) using a human insulin and insulin detemir standard curve. The data presented for insulin detemir are divided by eight to normalise for the injected dose.

Electroencephalography Telemetric EEG analysis was performed using implantable radio transmitters (PhysioTel transmitter TA10EA-F20; Data Sciences International [DSI], Lexington, MA, USA). All surgical procedures were conducted with the mouse under isoflurane (5% induction, 1.5% maintenance) anaesthesia. The mouse was placed in a stereotaxic head holder fitted with a mouse incisor bar. Midline skin incisions (10 mm on the head and 15 mm down the neck) were made and the s.c. tissue was bluntly separated. The body of the telemetry transmitter was implanted s.c. in a pouch made in the loose skin of the back and neck. The two lead wires were tunnelled s.c.

through the incision on the scull. Holes (0.7 mm diameter) were drilled in the cranium to implant two screws, around which the tips of the EEG leads were wrapped. For the recording electrode, the screws were placed epidurally 1 mm anterior to the lambda and 1 mm left of the sutura saggitalis, and for the reference electrode they were placed 1 mm anterior to the bregma and 1 mm right to the sutura coronaria. The electrodes and screws were then covered with dental acrylic cement, and the scalp sutured closely around the resulting wound with non-absorbable 5–0 suture material (Ethilon polyamide; Ethicon, Germany). Proper screw placement was confirmed histologically post mortem. The mice were given 1 week of post-surgical recovery before undergoing EEG recordings for further analysis. For in vivo stimulation, human insulin (1 U/kg body weight) or insulin detemir (2 U/kg body weight) was administered i.v. in the tail vein of awake, overnight-fasted mice in a cross-over design 5 days apart. After the injection, the mice were placed separately in their transparent home cages and an EEG was immediately recorded, lasting 30 min. Basal EEG data represent 1 h measurement in fed mice between 09.00 and 10.00 h when insulin was usually applied. Telemetry signals (EEG and motor activity) were processed by a Data-Sciences analog converter (Data Exchange Matrix; DSI) and stored digitally using the Dataquest A.R.T. 3.1 software (DSI). EEG activity was sampled at 250 Hz with a filter cut-off of 40 Hz. A video monitoring system was used in order to record referential EEGs and simultaneous video images showing the behaviour of the mice.

Data analysis for EEG measurements The acquired EEG data were imported into Somnologica Science software (version 3.3.1; Medcare, Reykjavík, Iceland) and power spectral analysis were calculated. Power spectral estimates provided the basic frequency characteristics of each recorded region. Consecutive 2-s epochs were subjected to a fast Fourier transform routine (Hanning smoothing window, each epoch divided into three 256-point overlapping segment windows with 50% overlap). Epochs of 2 s are to be preferred in intraoperative monitoring because they allow more rapid detection of change by increasing the statistical reliability of the power spectral estimate [21]. EEG power density (the square of the amplitude) was computed for a frequency range of 0.5–100 Hz (delta: 0.5–4 Hz, theta: 4–8 Hz, alpha: 8–12 Hz, beta: 12–30 Hz, and gamma: 30–100 Hz). The value of the EEG power density in each one of the different frequency bands was then examined and compared.

Statistical analysis Statistical analyses for Western blot analysis were done by a two-sided unpaired Student's *t*-test. Data in figures are expressed as means±SEM. The data from EEG measurements were analysed by a two-side paired Student's *t*-test.

Results

Metabolic effects and autophosphorylation of the IR and downstream elements in muscle tissue after stimulation with insulin detemir To determine whether the insulin analogue insulin detemir activates the insulin-signalling cascade to the same extent as human insulin, we injected either human insulin or insulin detemir into 12-week-old mice. Insulin administration in this study was performed by injection into the inferior vena cava to avoid the effect of altered s.c. absorption kinetics and to focus on direct

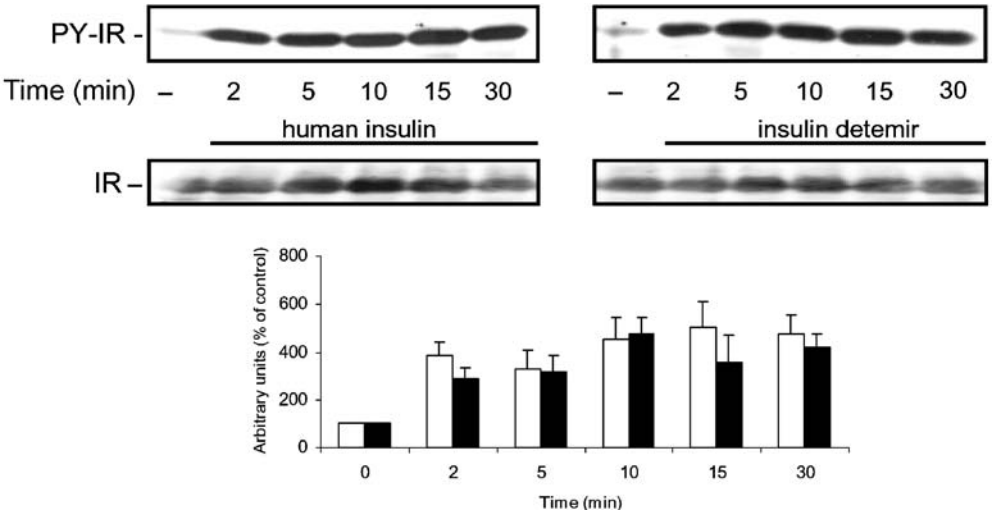

Fig. 1 Phosphorylation kinetics of the IR in muscle tissue. Mice were injected with either human insulin or insulin detemir into the inferior vena cava. Tissues were harvested after 2, 5, 10, 15 and 30 min and tyrosine phosphorylation was measured by immunoblotting in IR immunoprecipitates from muscle tissue. To ensure comparable loading, the membranes were blotted with anti-IR antibody (*lower blots*). In the bar graph, quantification of phosphorylation kinetics for human insulin (*open bars*) and insulin detemir (*solid bars*) by scanning densitometry are displayed. Scanning data obtained from three independent experiments are expressed as fold increase over basal (untreated) phosphorylation± SEM. *PY-IR* Phosphorylated IR

IR-signalling characteristics. Throughout the 30-min experiment, glucose levels significantly dropped from 9.7±0.3 to 3.5±0.2 mmol/l for human insulin and to 4±0.6 mmol/l for insulin detemir ($p<0.01$, $n=7$).

Following insulin injection, muscle tissue was dissected after 2, 5, 10, 15 and 30 min and Western blot analysis was performed in order to determine the tyrosine phosphorylation of the IR. An intense phosphorylation was observed after 2 min and preserved for 30 min (Fig. 1). Both insulins displayed comparable kinetics with no significant difference being observed between the two insulins (all $p>0.1$). At the level of PI 3-kinase (Fig. 2a) and phospho-Akt (Fig. 2b), insulin detemir showed results comparable with human insulin, which suggests an equal activation of the IR-signalling cascade in muscle tissue (all $p>0.1$).

Autophosphorylation of the IR and downstream elements in liver tissue after stimulation with insulin detemir To test whether IR activation in liver is altered, we further injected mice with either human insulin or insulin detemir and determined IR phosphorylation states in liver tissue. IR tyrosine phosphorylation could be clearly detected in mice stimulated with human insulin as well as in mice stimulated with insulin detemir as early as 2 min after injection, and statistical analysis revealed no significant difference between the two insulins (Fig. 3, all $p>0.1$). Moreover, insulin stimulates the activation of PI 3-kinase (Fig. 4a) and phospho-Akt (Fig. 4b) in liver, and the extent and time course were similar for both insulins over the 30-min period (all $p>0.1$), suggesting that peripheral insulin detemir receptor-signalling kinetics in vivo are not altered due to the attachment of the fatty acid chain.

Phosphorylation of the IR and Irs2 in hypothalamic tissue after stimulation with insulin detemir Recent studies revealed that dysregulation of IR signalling in the brain is associated with the development of obesity and diabetes. Since IR and Irs2 are expressed in the hypothalamus, we evaluated the IR-signalling characteristics in vivo after insulin injection.

The mice were stimulated with insulin and hypothalamic tissue was harvested after 2, 5, 10, 15 and 30 min. Phosphorylation of the IR was detected by Western blot analysis using anti-*p*-Tyr antibody. Concurrent immunoblots revealed an earlier and more pronounced IR phosphorylation for insulin detemir as compared with human insulin (Fig. 5a), favouring the idea that insulin detemir acts faster and more strongly in the brain.

Irs2 is essential for intact insulin signalling in the brain, as partially dysregulated Irs2 signalling is responsible for hyperphagia and obesity [17]. Therefore, we determined the tyrosine phosphorylation state of Irs2 in the hypothalamus of mice. After insulin injection, tissues were removed after 2, 5, 10, 15 and 30 min, and Western blot analysis was performed. These data suggest that the activation levels of Irs2 in mice treated with insulin detemir are significantly elevated compared with human insulin with a shift to an earlier activation of the insulin-signalling cascade for insulin detemir (Fig. 5b).

Phosphorylation of the IR in cortical tissue, EEG and total brain insulin concentrations following insulin detemir treatment In addition to insulin signalling in hypothalamic tissue we determined the impact of insulin detemir stimulation on tyrosine phosphorylation in cortical tissue.

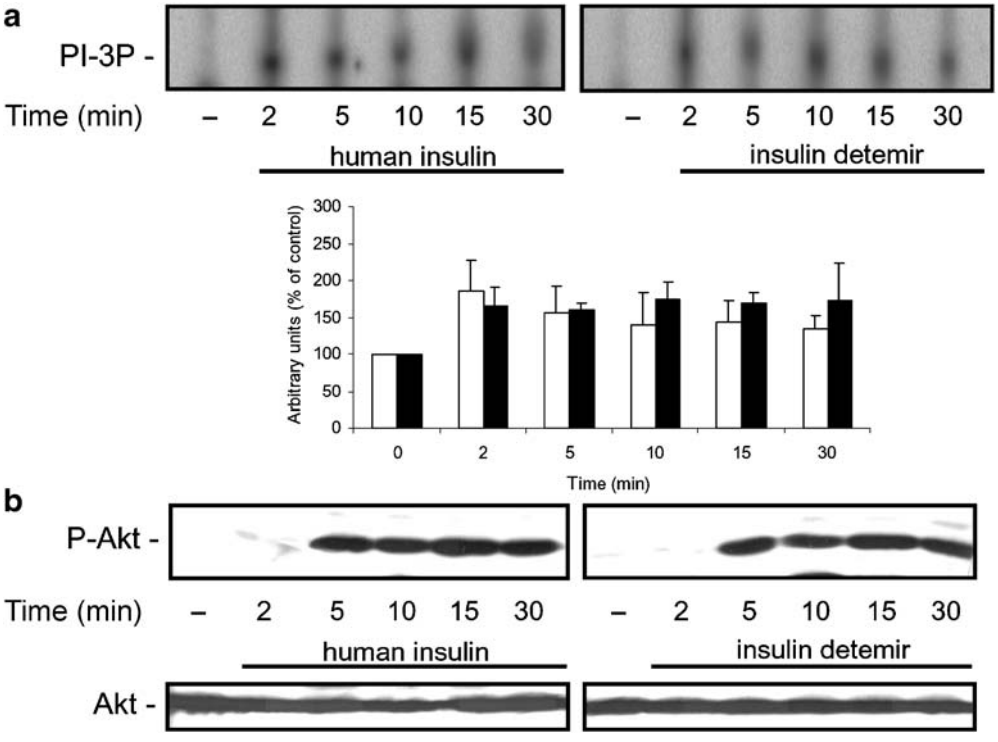

Fig. 2 Effect of insulin detemir on PI 3-kinase activity and phospho-Akt in muscle tissue. Mice were injected i.v. with either human insulin or insulin detemir. Muscle tissue was harvested at the indicated time points and lysates were immunoprecipitated with anti-p-Tyr antibody and an in vitro kinase assay was performed using L-α-phosphatidylinositol as a substrate (**a**). Scanning data for human insulin (*open bars*) and insulin detemir (*solid bars*) obtained from three independent experiments are expressed as fold increase over basal (untreated)±SEM. Lysates were immunoblotted with an anti-phospho-Akt antibody (**b**, *upper panel*), or anti-Akt antibody (**b**, *lower panel*). One of three individual experiments is shown. *P-Akt* Phosphorylated Akt, *PI-3P* phosphatidylinositol 3-phosphate

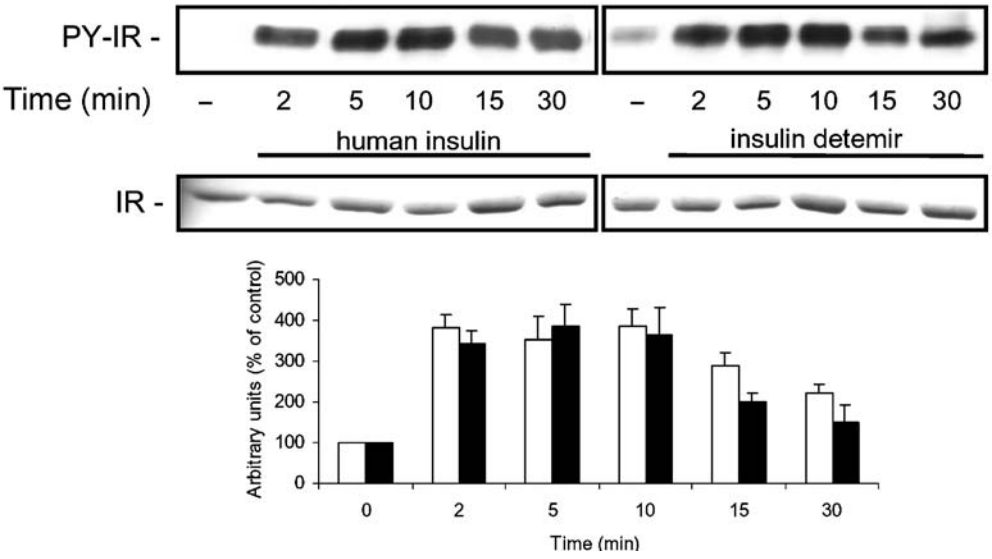

Fig. 3 Phosphorylation kinetics of the IR in liver tissue. Mice were injected i.v. with either human insulin or insulin detemir. Tissues were harvested at the indicated time points and tyrosine phosphorylation was measured by specific immunoblotting in IR immunoprecipitates from liver tissue. To ensure comparable loading the membranes were blotted with anti-IR antibody (*lower panel*). Quantification of IR autophosphorylation kinetics by scanning densitometry for human insulin (*open bars*) and insulin detemir (*solid bars*) is given in the bar graph. Scanning data obtained from three independent experiments are expressed as fold increase over basal (untreated) phosphorylation±SEM. *PY-IR* Phosphorylated IR

In agreement with the data obtained from the hypothalamus, IR phosphorylation was more enhanced by insulin detemir than by human insulin (Fig. 6a). To prove the fact that altered activation of the insulin-signalling cascade in cortical tissue is related to changes in brain activity, EEG recordings were obtained in the basal state and from mice that had been treated i.v. with either human insulin or insulin detemir in a cross-over design 5 days apart. Insulin detemir induced activation in delta, theta, alpha and beta EEG frequency activity (Fig. 6b). This suggests that as a consequence of improved cerebral insulin signalling, insulin detemir modulates electrical activity in the mouse cortex.

To determine the impact of an altered transport across the blood-brain barrier that might contribute to the brain data obtained, we measured total insulin concentrations in ethanol-acid brain extracts. Following i.v. injection, insulin detemir reached significantly higher concentrations

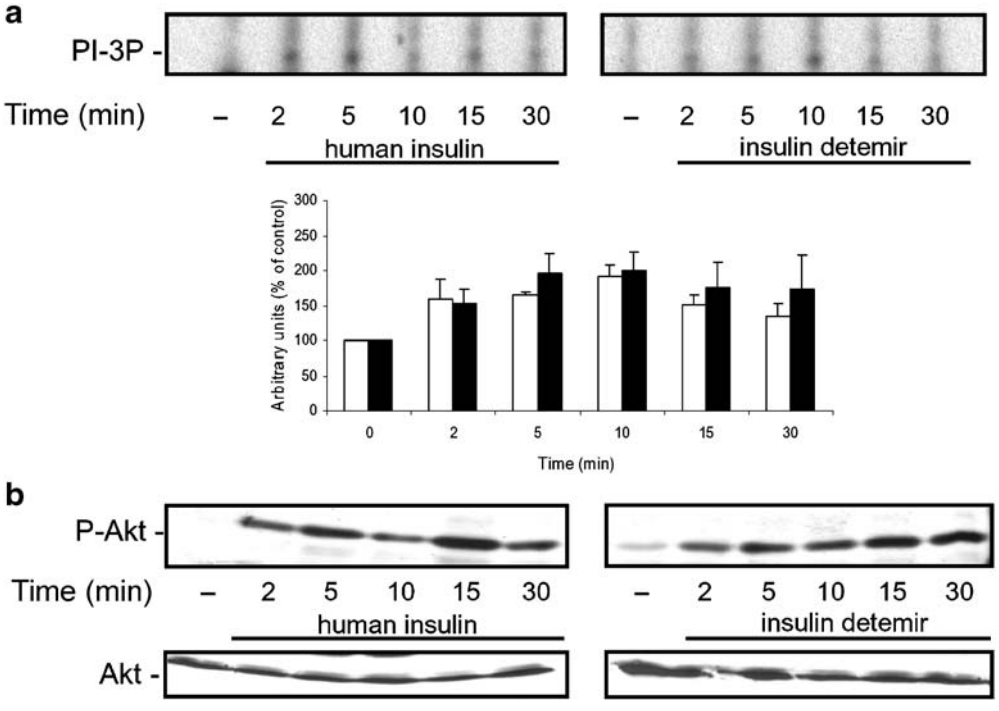

Fig. 4 Effect of insulin detemir on PI 3-kinase activity and phospho-Akt in liver tissue. Mice were injected i.v. with either human insulin or insulin detemir. Liver tissue was harvested at the indicated time points and lysates were immunoprecipitated with anti-p-Tyr antibody and an in vitro kinase assay was performed using L-α-phosphatidylinositol as a substrate (**a**). Scanning data for human insulin (*open bars*) and insulin detemir (*solid bars*) obtained from three independent experiments are expressed as fold increase over basal (untreated)±SEM. Lysates were immunoblotted with an anti-phospho-Akt antibody (**b**, *upper panel*), or anti-Akt antibody (**b**, *lower panel*). One of three individual experiments is shown. *P-Akt* Phosphorylated Akt, *PI-3P* phosphatidylinositol 3-phosphate

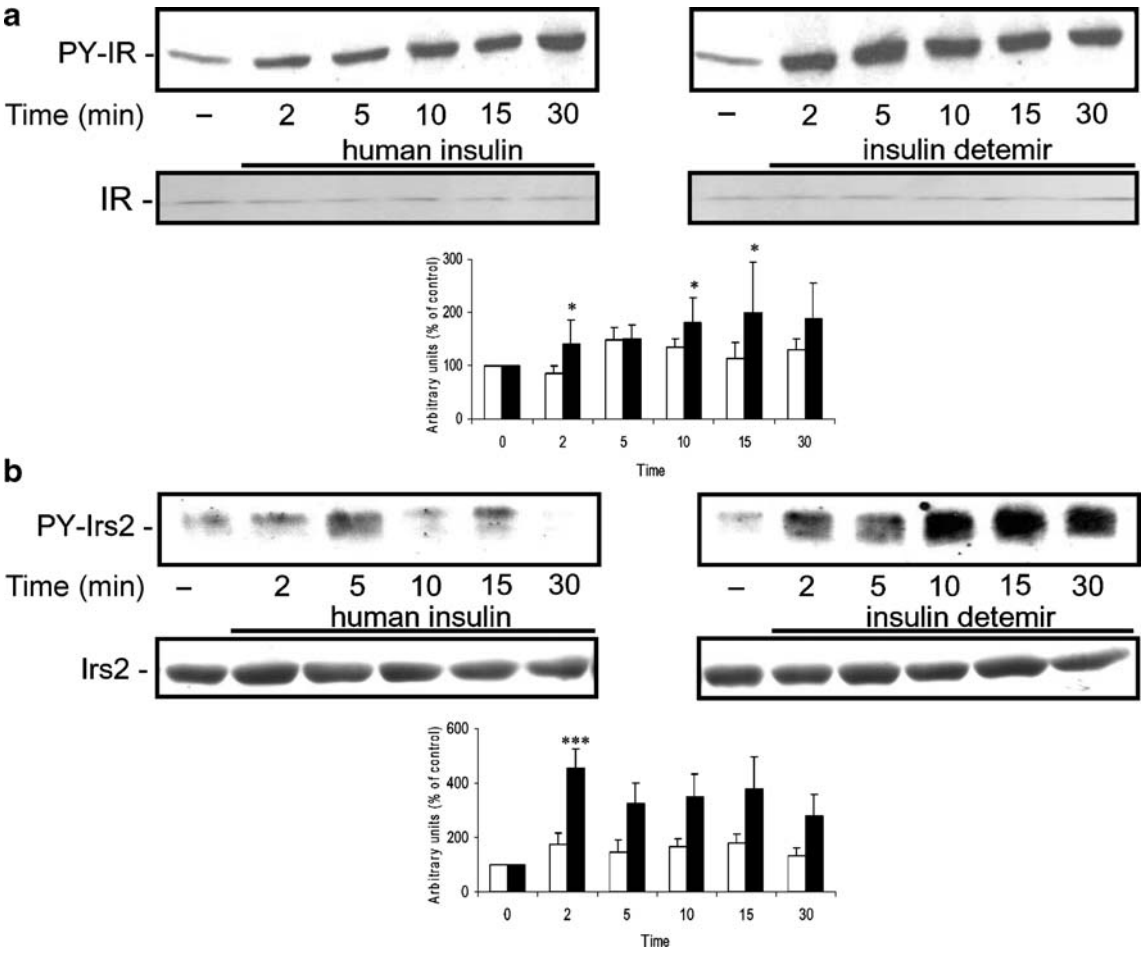

Fig. 5 Phosphorylation kinetics of the IR and Irs2 in hypothalamic tissue. Mice were injected i.v. with either human insulin or insulin detemir. Tissues were harvested at the indicated time points and tyrosine phosphorylation was measured by specific immunoblotting in IR (**a**) and Irs2 (**b**) immunoprecipitates. To ensure comparable loading the membranes were blotted with anti-IR and anti-Irs2 antibodies (*lower panels*). Quantification of IR (**a**) and Irs2 (**b**) tyrosine-phosphorylation kinetics for human insulin (*open bars*) and insulin detemir (*solid bars*) are given in the bar graphs. Scanning data obtained from six (IR) or eight (Irs2) independent experiments are expressed as fold increase over basal (untreated) phosphorylation±SEM. *$p<0.05$, ***$p<0.005$. *PY-IR* Phosphorylated IR, *PY-Irs2* phosphorylated Irs2

in the brain compared with human insulin (Fig. 6c, *$p<0.05$, **$p<0.01$, $n=5$).

We therefore conclude that treatment of mice with an acute i.v. dose of insulin detemir preferentially activates the cerebral insulin-signalling cascade while there is an equal IR activation in the periphery.

Discussion

Recombinant DNA technology has been used for almost two decades now to develop insulin analogues with advanced kinetic profiles and distinguished absorption [2]. Recently, the analogue insulin detemir was developed by the attachment of a 14-carbon fatty acid chain to LysB29 resulting in delayed absorption kinetics.

Here we report in vivo data from mice that were treated with either human insulin or the insulin analogue insulin detemir. In order to overcome the effect of altered s.c. absorption kinetics and to focus on direct IR-signalling characteristics, insulin was administrated by i.v. injection. Thereby, plasma concentrations immediately rose and a comparable onset of action and tissue distribution was achieved.

In our in vivo experiments, insulin detemir activates the insulin-signalling cascade in muscle and liver tissues to the same extent as human insulin does. However, in hypothalamic and cerebrocortical tissues, insulin-signalling kinetics determined by tyrosine-phosphorylation of the IR and Irs2 proteins were enhanced and the maximum was shifted to earlier time points, suggesting an altered profile of insulin detemir action in the cerebral nervous system.

As the time course and extent of activation of the insulin-signalling cascade in liver and muscle were comparable, we believe that insulin detemir action in the brain is enhanced due to the attachment of the fatty acid chain. This is reflected by elevated insulin detemir concentrations in the brain.

Although several mechanisms are suggested by which insulin may enter the brain from the blood, including

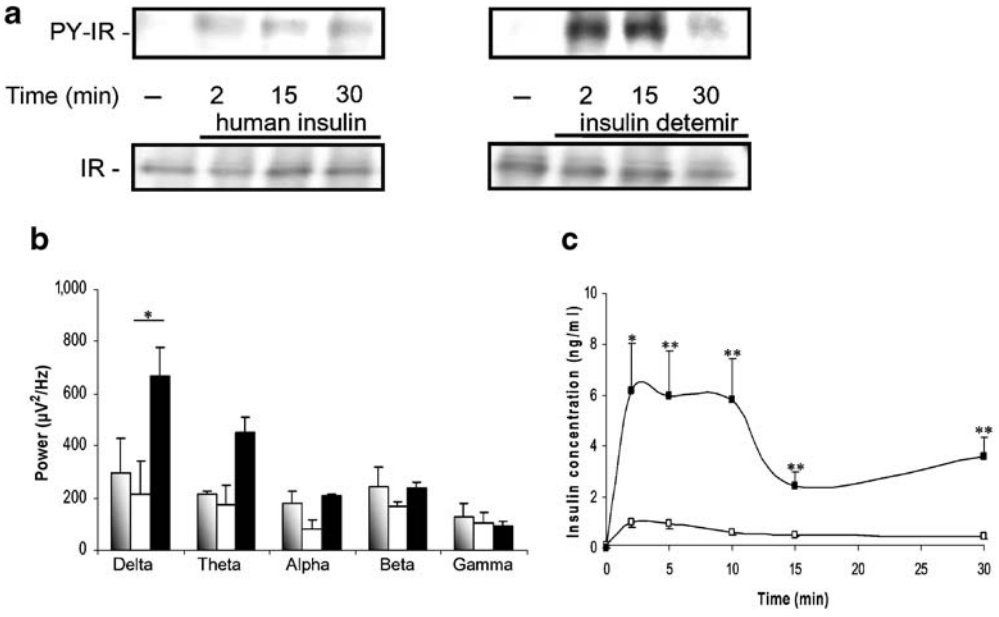

Fig. 6 Phosphorylation kinetics of the IR in cortical tissue and EEG analysis and total brain insulin concentrations following insulin detemir treatment. Mice were injected i.v. with either human insulin or insulin detemir. Cortical tissue was harvested at the indicated time points and tyrosine phosphorylation was measured by specific immunoblotting in IR immunoprecipitates (**a**). To ensure comparable loading the membranes were blotted with anti-IR antibody (*lower panel*). EEG power analysis of a 30-min post-injection period for human insulin (*white bars*) or insulin detemir (*black bars*) (**b**), means±SEM, n=2, *p<0.05. Delta: 0.5–4 Hz, theta: 4–8 Hz, alpha: 8–12 Hz, beta: 12–30 Hz and gamma: 30–100 Hz. The *grey bars* represent basal EEG power. **c** Insulin concentration in total brain following i.v. human insulin and insulin detemir injection, means± SEM, n=5, *p<0.05, **p<0.01. *PY-IR* Phosphorylated IR

receptor-mediated transport processes, conclusive data are not available [22]. However, it is known that albumin directly penetrates into the cerebrospinal fluid across choroids plexus epithelial cells [23], and there is evidence that as lipophilicity and membrane permeability of a molecule increases, the concentration in the cerebrospinal fluid rises [24]. Moreover, one could speculate that in the presence of a low albumin concentration such as in the cerebrospinal fluid, the rate of unbound insulin detemir is increased, and therefore an enhanced amount of 'active' insulin detemir molecules is available to bind to the IR. In addition, the impact of the fatty acid chain on IR binding in the brain is unknown; however, it could indeed also count towards enhanced IR signalling.

The observation of enhanced insulin signalling in the brain using insulin detemir is of particular interest in the context of former studies in humans using intranasal insulin, which enters the cerebrospinal fluid compartment without affecting circulating insulin levels in the bloodstream. In these healthy subjects the administration of nasal insulin led to a loss of body weight and body fat over an 8-week treatment accompanied by a drop in plasma leptin levels. These data provide a profound basis for a negative feedback signal of insulin in the regulation of body weight and adiposity [25]. Moreover, a recent study in humans compared the effect of human insulin and insulin detemir on hepatic glucose production [26]. While the glucose infusion rate and glucose disposal were similar after s.c. injection, hepatic glucose production within a 16-h euglycaemic clamp was increased using insulin detemir. These data are somehow contradictory to our in vivo data on equal IR signalling in liver tissue; however, the altered observation time (short-term vs long-term) and the route of administration (s.c. vs i.v.) might be responsible for these findings.

On a molecular basis, impressive data from mouse models have accumulated over the past years suggesting that dysregulation of insulin action at the level of the IR and downstream signalling targets in the central nervous system is associated with obesity and diabetes [27, 28]. The precise underlying mechanisms are uncertain and difficult to establish, but it is known that female mice lacking the IR specifically in the brain display increased food intake, and both male and female mice developed diet-sensitive obesity with elevated body fat and plasma leptin levels including mild insulin resistance. Furthermore, experimental desensitisation of the cerebral IR resulted in both a cognitive deficit and abnormalities in cerebral oxidative glucose metabolism [29], supporting the idea that intact insulin signalling in the brain is a pivotal element for brain function and glucose homeostasis. At the Irs level, Irs2 is potentially important since a lack of Irs2 in the hypothalamus results in increased appetite and body mass, leading to insulin resistance and finally diabetes [17]. Moreover, the loss of Irs2 in the mouse brain is associated with reduced neuronal proliferation during development, as well as accumulation of neurofibrillary tangles containing phosphorylated tau in the hippocampus, favouring intact

Irs2 signalling in the hypothalamus as neuroprotective [30]. This is in agreement with a study in humans showing that intranasal administration of insulin leads to an improvement of cognitive function and memory, opening a whole new aspect in insulin therapy [25].

At the level of PI 3-kinase, it has been demonstrated that the food-intake-lowering effect of insulin in mice is reversed by intracerebroventricular infusion of PI 3-kinase inhibitors [31], further supporting the idea that the Irs-PI 3-kinase pathway is a strong mediator of appetite and weight control.

Since the impact of an earlier and increased tyrosine phosphorylation of the IR and Irs2 in hypothalamic tissue observed during insulin detemir treatment remains to be further elucidated, it can be suggested that pronounced activation of the insulin-signalling cascade in the brain is sufficient to prevent hyperphagia and weight gain in insulin detemir therapy. If this is the case, effects obtained in this study might be related to the differences in weight development observed in clinical trials comparing insulin detemir with NPH insulin treatment.

Moreover, enhanced cortical EEG delta and theta activity represent the control of neuronal plasticity, and presumably learning and reprocessing of memories [32, 33].

Taken together, the current in vivo data suggest that the novel insulin analogue insulin detemir is characterised by an increased rate of brain action vs peripheral action. This characteristic profile of insulin detemir may provide a new concept for insulin therapy in obese people with diabetes.

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References

1. Mudaliar SR, Lindberg FA, Joyce M et al (1999) Insulin aspart (B28 asp-insulin): a fast-acting analog of human insulin: absorption kinetics and action profile compared with regular human insulin in healthy nondiabetic subjects. Diabetes Care 22:1501–1506
2. Gerich JE (2002) Novel insulins: expanding options in diabetes management. Am J Med 113:308–316
3. Gerich JE (2004) Insulin glargine: long-acting basal insulin analog for improved metabolic control. Curr Med Res Opin 20:31–37
4. Havelund S, Plum A, Ribel U et al (2004) The mechanism of protraction of insulin detemir, a long-acting, acylated analog of human insulin. Pharm Res 21:1498–1504
5. Kurtzhals P, Havelund S, Jonassen I, Markussen J (1997) Effect of fatty acids and selected drugs on the albumin binding of a long-acting, acylated insulin analogue. J Pharm Sci 86: 1365–1368
6. Kurtzhals P (2004) Engineering predictability and protraction in a basal insulin analogue: the pharmacology of insulin detemir. Int J Obes Relat Metab Disord 28(Suppl 2):S23–S28
7. Kurtzhals P, Schaffer L, Sorensen A et al (2000) Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use. Diabetes 49:999–1005
8. Kurtzhals P, Havelund S, Jonassen I, Kiehr B, Ribel U, Markussen J (1996) Albumin binding and time action of acylated insulins in various species. J Pharm Sci 85:304–308
9. De Leeuw I, Vague P, Selam JL et al (2005) Insulin detemir used in basal-bolus therapy in people with type 1 diabetes is associated with a lower risk of nocturnal hypoglycaemia and less weight gain over 12 months in comparison to NPH insulin. Diabetes Obes Metab 7:73–82
10. Heise T, Nosek L, Ronn BB et al (2004) Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes. Diabetes 53:1614–1620
11. Vague P, Selam JL, Skeie S et al (2003) Insulin detemir is associated with more predictable glycemic control and reduced risk of hypoglycemia than NPH insulin in patients with type 1 diabetes on a basal-bolus regimen with premeal insulin aspart. Diabetes Care 26:590–596
12. Wing RR, Klein R, Moss SE (1990) Weight gain associated with improved glycemic control in population-based sample of subjects with type I diabetes. Diabetes Care 13:1106–1109
13. Bailey CJ, Grant PJ (1998) The UK Prospective Diabetes Study. Lancet 352:1932
14. Fritsche A, Haring H (2004) At last, a weight neutral insulin? Int J Obes Relat Metab Disord 28(Suppl 2):S41–S46
15. White MF (2002) IRS proteins and the common path to diabetes. Am J Physiol Endocrinol Metab 283:E413–E422
16. Pirola L, Johnston AM, Van Obberghen E (2004) Modulation of insulin action. Diabetologia 47:170–184
17. Lin X, Taguchi A, Park S et al (2004) Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. J Clin Invest 114:908–916
18. Porte D Jr, Baskin DG, Schwartz MW (2005) Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. Diabetes 54:1264–1276
19. Hennige AM, Burks DJ, Ozcan U et al (2003) Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetes. J Clin Invest 112:1521–1532
20. Kellerer M, Koch M, Metzinger E, Mushack J, Capp E, Haring HU (1997) Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK- 2) and insulin receptor substrate-2 (IRS-2) dependent pathways. Diabetologia 40:1358–1362
21. Levy WJ (1987) Effect of epoch length on power spectrum analysis of the EEG. Anesthesiology 66:489–495
22. Woods SC, Seeley RJ, Baskin DG, Schwartz MW (2003) Insulin and the blood–brain barrier. Curr Pharm Des 9:795–800
23. Knott GW, Dziegielewska KM, Habgood MD, Li ZS, Saunders NR (1997) Albumin transfer across the choroid plexus of South American opossum (Monodelphis domestica). J Physiol 499:179–194
24. Shen DD, Artru AA, Adkison KK (2004) Principles and applicability of CSF sampling for the assessment of CNS drug delivery and pharmacodynamics. Adv Drug Deliv Rev 56:1825–1857
25. Benedict C, Hallschmid M, Hatke A et al (2004) Intranasal insulin improves memory in humans. Psychoneuroendocrinology 29:1326–1334
26. Hordern SV, Wright JE, Umpleby AM, Shojaee-Moradie F, Amiss J, Russell-Jones DL (2005) Comparison of the effects on glucose and lipid metabolism of equipotent doses of insulin detemir and NPH insulin with a 16-h euglycaemic clamp. Diabetologia 48:420–426

27. Burks DJ, de Mora JF, Schubert M et al (2000) IRS-2 pathways integrate female reproduction and energy homeostasis. Nature 407:377–382
28. Bruning JC, Gautam D, Burks DJ et al (2000) Role of brain insulin receptor in control of body weight and reproduction. Science 289:2122–2125
29. Henneberg N, Hoyer S (1995) Desensitization of the neuronal insulin receptor: a new approach in the etiopathogenesis of late-onset sporadic dementia of the Alzheimer type (SDAT)? Arch Gerontol Geriatr 21:63–74
30. Schubert M, Brazil DP, Burks DJ et al (2003) Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. J Neurosci 23:7084–7092
31. Niswender KD, Morrison CD, Clegg DJ et al (2003) Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. Diabetes 52:227–231
32. Selbach O, Doreulee N, Bohla C et al (2004) Orexins/hypocretins cause sharp wave- and theta-related synaptic plasticity in the hippocampus via glutamatergic, gabaergic, noradrenergic, and cholinergic signaling. Neuroscience 127:519–528
33. Molle M, Marshall L, Gais S, Born J (2004) Learning increases human electroencephalographic coherence during subsequent slow sleep oscillations. Proc Natl Acad Sci USA 101: 13963–13968