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Selective delivery of insulin into the brain: intraolfactory absorption

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Abstract

The distribution of insulin between blood and brain was investigated in mice. The drug was administered subcutaneously and by instilling the drug to the olfactory region of the nasal cavity. The concentrations of insulin in the biological samples were measured by a γ-counter. A significantly higher concentration of insulin was measured in the brain, following intraolfactory administration compared to subcutaneous injection. The absorption was also found to be very rapid. Ten minutes after the administration the concentration in the brain had reached 193 counts/min per g (equivalent to 37 μU insulin/g tissue), which was 487% higher than achieved after subcutaneous injection. The majority (> 99%) of the CNS drug development programmes are devoted solely to CNS drug discovery and less than 1% are devoted to drug delivery. The results suggest that it may be possible to achieve absorption directly into the brain, by-passing the blood–brain barrier. The olfactory region may be the key for this absorption.

1. Introduction

There is a common belief that only if molecules are small enough will they be able to cross the blood–brain barrier (BBB). This is partly true; the presence of this physiological bottleneck means that circulating molecules gain access to the brain via only those pathways or criteria made by this highly resistance barrier. The BBB is able to minimize the transport of most potent neuropharmaceuticals and hormones, such as insulin, from blood to brain and the permeability barrier of BBB is able to eliminate both para- and transcellular movement into the brain. More than 99% of existing central nervous system (CNS) drug development programmes are devoted solely to CNS drug discovery, primarily the discovery of small lipophilic molecules, and less than 1% are devoted to CNS drug delivery (Pardridge, 1995).

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Until some years ago, researchers believed that circulating insulin was incapable of crossing the BBB and, therefore, without effect in the brain (Schwartz et al., 1992). Recent investigations, however, indicate the opposite. Insulin in the central nervous system is largely derived from the circulation and the entry into the brain is facilitated by a saturable transport process (Schwartz et al., 1992; Baura et al., 1993). Studies on the pharmacology of insulin in the CNS have, therefore, been expanding rapidly in the past decade. It has now been suggested that trans-endothelial transport across the BBB and the concentration of insulin in the brain may play a key role in the development of obesity (Schwartz et al., 1992). This is controversial with the common belief that obesity is frequently associated with a sedentary life-style, inactivity and excessive caloric intake (Schwartz et al., 1992; Mei et al., 1993; Proietto and Thorburn, 1994). Insulin infusion, administered into the hypothalamic area or the cerebral ventricles of the brain, have been shown to cause a reliable and predictable decrease in food intake and weight loss (Schwartz et al., 1995; Van Dijk et al., 1995). Recent studies on obese rats and baboons have shown that the animals lose weight when treated with intrathecal infusion of insulin (Baskin et al., 1988; Mei et al., 1993). Therefore, pathological conditions, such as decreased trans-endothelial transport of insulin across the BBB or decreased insulin production due to diabetes (especially type II diabetes), may be the cause of adiposity. While parenteral administration of insulin results in increased food intake, intrathecal administration of low dose insulin shows the opposite effect (Baskin et al., 1988; Mei et al., 1993). The development of new drug delivery systems are needed, providing selective delivery of insulin into the brain. Such systems may be the key to the treatment of diseases associated with hypoinsulinemia in the brain.

It has been shown that substances, such as ^{32}P (as phosphoric acid), colloidal ^{198}Au and Cd^{2+}, may be found in the cerebrospinal fluid, if they are injected under the mucosal membrane at the olfactory region (Czerniawska, 1970; Evans and Hastings, 1992). Studies on *vesicular stomatitis virus* have shown that the virus is able to cross the olfactory region, into the brain, through the olfactory fibres (Lundh et al., 1988). These results indicate that drugs may be transported across the olfactory region and directly into the brain, bypassing the BBB. In the last two decades, the intranasal route has gained much attention for insulin therapy for diabetes mellitus as well as other drug treatments, due to the thin epithelial membrane in the nasal cavity and the extensive network of blood capillaries provide an optimal surrounding for successful absorption (Gizurarson and Bechgaard, 1991a; Gizurarson, 1993). No studies, however, are found in the literature, where the olfactory region is described as an entry into the brain, providing a pathway for drugs, which normally do not cross the BBB. The olfactory system is the organ that animals use to sample and detect chemical nature, whether hostile or hospitable, aqueous or gaseous, above or below the ground, dry or wet. The olfactory organs are lined with olfactory receptor cells (both ciliated cells and cells having microvilli) which are surrounded by microvillus supporting cells (Menco, 1992a). The mucus layer, covering the olfactory epithelium, is able to dissolve various molecules, prior to reaching the olfactory organ (Getchell and Getchell, 1992), whereafter the message is transduced to the rest of the cell and into the brain via the olfactory cilia and the receptors located on the ciliary membrane (Menco, 1992b).

The aim of this study is to evaluate whether insulin may be administered selectively, into the brain, using the olfactory region of the nasal cavity as an entry and measuring the absorption rate and the proportion absorbed into the brain.

2. Materials and methods

2.1. Chemicals

Human insulin and ^{125}I-insulin (mono-^{125}I-(Tyr A14) human insulin) (28 μCi/ml) were kindly provided by Novo Nordisk A/S (Bagsvaerd, Denmark). The solution of ^{125}I-Insulin in distilled water was used as received. Pentobarbital was purchased from Lyfjaverslun Islands (Reykjavik, Iceland) and propylenglycol from Norsk Medisi-

naldepot (Oslo, Norway). All other substances were of analytical grade.

2.2. Animals

Male and female Balb/c mice (15–20 g) were obtained from Keldur (Institute for Experimental Pathology, University of Iceland, Reykjavik, Iceland) and used in all experiments. The animals had not been used in other experiments prior to the study.

2.3. Formulations and administration procedures

The insulin formulation for intraolfactory administration (i.o.) contained 0.76 mU/μl unlabelled and 21 nCi/μl ^{125}I labelled insulin. Each mouse received 7 μl. Four techniques were used for the delivery to the olfactory region:

Method I: The experimental procedure was performed by injection through the palate into the olfactory region, as described in Gizurarson et al. (1995).

Method II: The insulin was administered on the nostrils, while the mice were fixed in a supine position, allowing them to inhale the solution into the nasal cavity.

Method III: The insulin was instilled into the olfactory region, using a polyethylene tube (d = 0.25 μm), cannulated 5 mm into the nasal cavity, to the olfactory region. The tube was fixed on a modified pipette tip, as described in Gizurarson et al. (1995).

Method IV: The insulin was instilled as described in Method III, except for the length of the tube, which was now cannulated 2 mm into the nasal cavity toward the olfactory region.

The insulin formulation for subcutaneous injection (s.c.) was prepared by diluting the i.o. formulation with saline to a final concentration of 26.55 mU/ml unlabelled and 733.5 nCi/ml ^{125}I-labelled insulin. Each mouse received 200 μl.

At 10 and 15 min after the i.o. and s.c. administration, respectively, the animals were sacrificed. Full blood was collected by heart puncture and the brain was removed within 2 min postmortum. The weight of each sample was recorded.

2.4. Pharmacokinetic studies

Seventy mice were divided into 14 groups of 5 mice each. Six mice were used as controls, receiving no drug, but the rest received insulin and ^{125}I-insulin either s.c. or by i.o. as described in Method I. At 5, 10, 15, 30, 45 and 60 min after the administration, the animals were sacrificed. Samples from blood, brain, liver, spleen and the stomach were collected and the insulin concentration was measured.

2.5. Analysis, calculations and statistics

The concentration of insulin in each sample was measured by means of a γ-counter (GammaCounter NE1612, Neuclear Enterprise, UK) and expressed in counts/min per g organ. For the calculation of the insulin concentration, the radioactivity was calculated relative to a standard curve for labelled insulin and expressed in μU/g organ. The detection limit was 0.25 nCi. The results were corrected for dose and adjusted so that 10 mU insulin per dose had been injected to each animal. Standard statistical methods were used throughout the study. The results were expressed as mean $\pm$ 95% confidence limit.

3. Results and discussion

3.1. Evaluation of administration techniques

Results from various intranasal techniques show that small modifications in the administration technique affect the bioavailability, significantly (Gizurarson et al., 1996). In this study, results from the evaluation of four administration techniques are shown in Fig. 1a and the respective brain/blood ratio in Fig. 1b. They show that Method I (through the palate), was found to be the best technique, providing approximately four times higher insulin concentration in the brain, compared with the same dose administered s.c. Methods I–IV were, however, all able to increase the brain/blood ratio above the value achieved after s.c. injection, indicating that the relative amount absorbed into the brain is significantly

higher than the amount transported from the peripheral system into the brain. But Method I was the only one that gave reproducible and clinically relevant absorption of insulin into the brain.

3.2. Pharmacokinetics

Circulating insulin molecules gain access to the brain interstitial fluid via a receptor-mediated transport mechanism. This transport, however, takes minutes to occur and involves sequential steps of receptor-mediated endocytosis at the lumenal plasma membrane of the endothelial cell, followed by transcytosis through an endosomal subcellular structure within the endothelial cell and finally exocytosis at the ablumenal membrane into the interstitial fluid of the brain (Pardridge, 1995). This mechanism may easily be affected by various factors, resulting in hypoinsulinemia inside the brain. Instead of focusing on new molecules, which may increase the transport of insulin across the BBB, this work is devoted to bypassing the BBB, using new drug delivery systems.

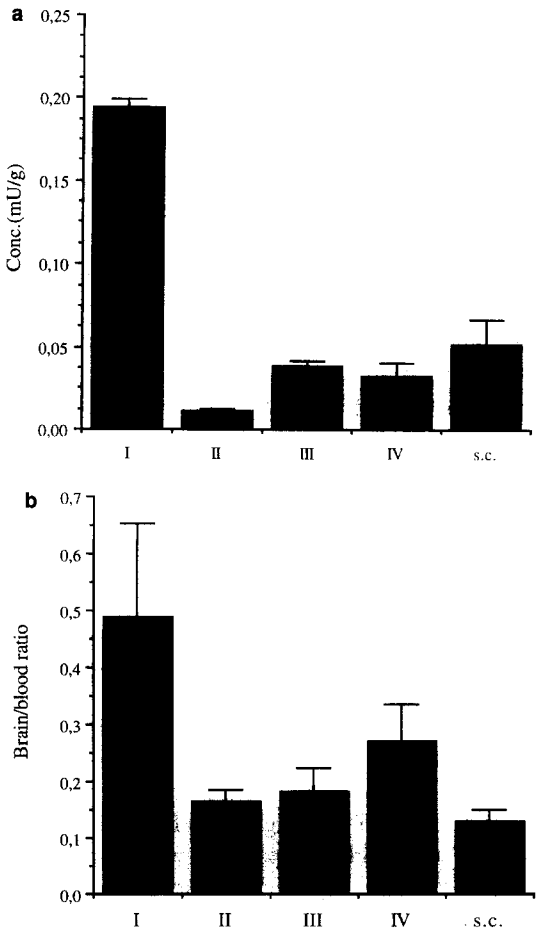

Fig. 1. The insulin concentration in the brain (a) and the brain/blood ratio (b) after five different administration techniques, in mice. Four techniques for intranasal/intraolfactory administration (I–IV) were evaluated as well as one subcutaneous injection (s.c.).

Contrary to peripherally absorbed insulin, intraolfactory delivery of insulin resulted in a significant absorption of the hormone into the brain as soon as 10–15 min after the instillation. After 10–15 min the count (counts/min) detected represents insulin molecules (Fig. 2a). After 30 min, a high percentage of the counts is probably degraded insulin and therefore does not represent correct amounts of insulin. Previous results have demonstrated that insulin is not degraded in the nasal fluid and may therefore cross into the brain in intact form (Gizurarson and Bechgaard, 1991b) and the ^{125}I is not removed from the insulin molecule, unless it is degraded intracellularly. Ten minutes after the administration, the concentration in the brain had reached 193 counts/min per g (equivalent to 37 μU/g), but for the subcutaneous group the concentration was only 40 counts/min per g (equivalent to 13 μg/g). Fifteen minutes after the application, the i.o. delivery showed about 4.1 times higher concentration in the brain, than was seen after s.c. injection and the absorption rate (k_{abs}) was found to be 2.3 μU/g per min and 1.0 μU/g per min, for the i.o. and s.c. administration, respectively, and the relative bioavailability (F_{rel} (i.o./s.c.)) was 370%. The concentration–time profile in the blood, however, was not distinguishable between these two routes, as shown in Fig. 2b.

Formulations, instilled into the olfactory region, will partly be transported to the respiratory area and be absorbed into the systemic circulation. This is seen from Fig. 2b, where the systemic absorption after io administration is very rapid during the first 15 min. However, the blood in-

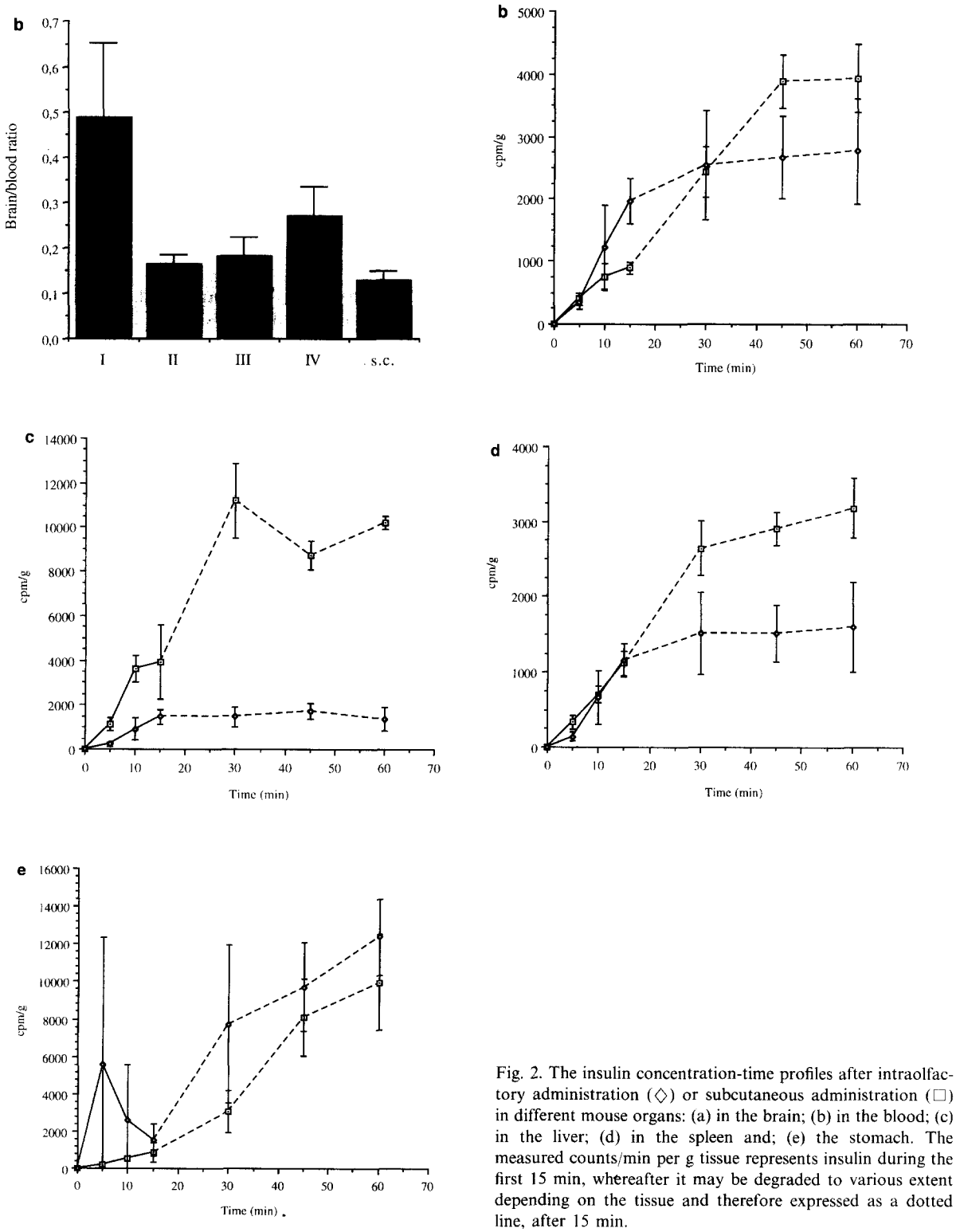

Fig. 2. The insulin concentration-time profiles after intraolfactory administration (◇) or subcutaneous administration (□) in different mouse organs: (a) in the brain; (b) in the blood; (c) in the liver; (d) in the spleen and; (e) the stomach. The measured counts/min per g tissue represents insulin during the first 15 min, whereafter it may be degraded to various extent depending on the tissue and therefore expressed as a dotted line, after 15 min.

sulin levels after s.c. injection were increasing throughout the study. Insulin concentration in the liver and spleen are shown in Fig. 2c,d, respectively. Significantly more amounts of peripherally injected insulin are found in the liver, 5 min after the administration than after intraolfactory administration. The ^{125}I content in the liver may demonstrate that the relative bioavailability (F_{rel} (i.o./s.c.)) after these two routes is about 28%. A similar pattern was also seen in the spleen, but the relative amount absorbed into the spleen is much higher (F_{rel} (i.o./s.c.) = 86%) than into the liver.

Samples from the stomach show that significant amounts of insulin have transported from the nasal cavity to the pharynx and swallowed into the stomach, as soon as 5 min after i.o. administration (Fig. 2e). However, rapid accumulation of counts/min was seen in the stomach during the study which is in the agreement with the fact that ^{125}I, after it has been degraded from the insulin molecule, is able to undergo hydrochloric acid metabolism in a similar matter as chlorine and secreted into the stomach (Jensen et al., 1991).

Previous results (unpublished), using diazepam as a model drug, showed that 10 min after the application the highest drug concentration was not found in the olfactory bulb, as expected, but just behind the olfactory bulb. The experiment was conducted in such a way that 10 min after the administration of diazepam, the brain was removed and divided into 6 slices where the olfactory bulb was measured separately. The results indicate that the drug had passed through the olfactory bulb and was continuing through the olfactory tract to the thalamus or to the limbic system. Further studies are in progress, exploring the distribution of insulin as well as other drugs in the brain after i.o. or s.c. administration.

The results show that the olfactory region may represent a pathway for drugs that do not have the right characteristics or are to big to be able to negotiate with the BBB and be transported to the brain. A significant amount of insulin was rapidly absorbed into the brain, which support this idea. Further studies are in progress, looking at various physiological and pharmaceutical factors that may affect this transport mechanism. Future experiments will help us to answer the question, whether this route may be clinically relevant or not. Our studies show that the route may provide a new possibility for drugs that would be appreciated in the pharmacotherapy of CNS diseases, but are not applicable due to the highly resistant and selective BBB.

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