Non-islet cell tumour-induced hypoglycaemia: a review of the literature including two new cases

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Abstract

This review focuses on the tumour types and symptoms associated with non-islet cell tumour-induced hypoglycaemia (NICTH) as well as the pathogenesis, diagnosis and treatment of this rare paraneoplastic phenomenon. In addition, we report two illustrative cases of patients suffering from NICTH caused by a solid fibrous tumour and a haemangiopericytoma respectively. In the first case, NICTH resolved following complete resection of the tumour, but in the second case the patient needed long-term treatment aimed at controlling hypoglycaemia because of non-resectable metastases. Many tumour types have been associated with NICTH. The crucial event in the development of NICTH seems to be overexpression of the IGF-II gene by the tumour. NICTH is characterised by recurrent fasting hypoglycaemia and is associated with the secretion of incompletely processed precursors of IGF-II (‘big’-IGF-II) by the tumour. This induces dramatic secondary changes in the circulating levels of insulin, GH, IGF-I and IGF-binding proteins, resulting in an insulin-like hypoglycaemic activity of ‘big’-IGF-II.

Introduction

Hypoglycaemia is a common medical emergency, mostly as a result of a complication of therapy with insulin and/or oral hypoglycaemic agents in diabetes mellitus. In rare cases, hypoglycaemia can be a manifestation of neoplastic disease. Tumours related to the occurrence of hypoglycaemia can, as a general rule, be divided into three groups. First, tumours can produce excess insulin such as pancreatic insulinomas or ectopic insulin-producing tumours. Second, hypoglycaemia can be caused by tumour-related factors such as destruction of the liver and adrenal glands by massive tumour infiltration. Finally, hypoglycaemia rarely can be induced by the production of substances interfering with glucose metabolism including insulin receptor antibodies (in Hodgkin’s disease and other haematological malignancies; Marks & Teale 1998), various cytokines including tumour necrosis factor-α and interleukin-1 and -6 (Marks & Teale 1998, Lang et al. 2001), catecholamines (in phaeochromocytomas), secretion of insulin-like growth factor (IGF)-I (Nauck et al. 2007) and tumours that secrete partially processed precursors of IGF-II (‘big’-IGF-II; Service 1995, Marks & Teale 1998). The latter condition is also known as non-islet cell tumour-induced hypoglycaemia (NICTH).

This review will focus on NICTH. So far, the literature on this rare and complex biochemical syndrome involving many types of tumours is mostly limited to case reports. For this reason, besides a brief description of two new cases of NICTH, this paper reviews the available literature on this paraneoplastic phenomenon with respect to its pathogenesis, diagnosis and treatment.
The IGF system

IGF physiology

The IGF system is composed of two IGF ligands (IGF-I and IGF-II) and two IGF receptors (the IGF-I receptor (IGF1R) and the IGF-II/manose-6-phosphate-receptor (IGF2R); Pollak et al. 2004). The majority of circulating IGF-I and IGF-II are produced by the liver, although various tissues and cell types are also capable of synthesising these peptides. The synthesis of IGF-I by the liver and various other organs largely depends on its stimulation by growth hormone (GH) through the GH receptor, whereas the synthesis of IGF-II is relatively independent of GH action. The GH/IGF-I axis is the primarily regulator of postnatal growth while IGF-II appears to have an important role during foetal development, cell proliferation and apoptosis (Jones & Clemmons 1995, van Buul-Offers 1996, LeRoith 1997).

Both IGF-I and IGF-II are structurally and functionally related to insulin. Most of the biological actions of IGF-I and IGF-II are thought to be mediated via IGF1R as reviewed extensively by others (LeRoith 1997, Rajaram et al. 1997, O’Dell & Day 1998, Dupont et al. 2003, Denley et al. 2005, Hartog et al. 2007). However, IGFs may also interact with the insulin receptor which contributes to the pleiotropic nature of IGF activity in the body.

The IGF1R is a tyrosine kinase receptor consisting of two extracellular ligand binding α-subunits and two β-subunits comprising the transmembrane and tyrosine kinase domains (Hubbard & Miller 2007). IGF1R signalling can induce differentiation, malignant transformation and regulate cell–cell adhesion (LeRoith 1997, Rajaram et al. 1997, O’Dell & Day 1998, Dupont et al. 2003, Denley et al. 2005, Hartog et al. 2007). However, IGFs may also interact with the insulin receptor which contributes to the pleiotropic nature of IGF activity in the body.

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Due to alternative splicing of exon 11 of the insulin receptor gene, there are two isoforms of the insulin receptor (isoforms A and B). The insulin receptor-A isoform is preferentially expressed in foetal tissues and in certain human malignancies, whereas the insulin receptor-B isoform is mainly expressed in important target tissues for the metabolic effects of insulin including liver, muscle and fat. IGF-I has low affinity for these receptors, but IGF-II binds with high affinity (comparable with its affinity for IGF1R) and activates isoform A (Frasca et al. 1999). This interaction leads predominantly to mitogenic effects (Frasca et al. 1999, Sciacca et al. 2002, Belfiore 2007). In contrast, the low-affinity binding of IGF-II to the B-isoform of the insulin receptor results in insulin-like metabolic effects.

Insulin and IGF-I receptors are structurally homologous – both exhibit intrinsic tyrosine kinase activity and in part interact with various similar intracellular signal transduction mediators (Phillips & Robertson 1993, Baxter 1996, LeRoith 1997). The structural homogeneity allows formation of hybrid receptors of IGF1R and insulin receptor-A or -B. When IGF1R and insulin receptor are co-expressed on the same cell, receptor hybrids form by random assembling and the least abundant receptor is drawn predominantly into hybrid receptors (Siddle et al. 2001). IGF-II can bind these hybrid receptors with high affinity but the biological role of these hybrid receptors remains largely unknown.

In addition, IGF-II also binds to the IGF2R. Besides its role in the transport of lysosomal enzymes from the Golgi-apparatus to the lysosomes, this receptor is thought to function primarily as a scavenger receptor, promoting the endocytosis and degradation of extracellular IGF-II, thus regulating local IGF-II levels (Jones & Clemmons 1995, LeRoith 1997).

The glucose-lowering effect of IGFs is ~10 times lower than that of insulin, but in healthy subjects the serum concentration of IGFs is about 1000 times higher than insulin (Rinderknecht & Humbel 1978a,b). However, in contrast to insulin and proinsulin, in the circulation most (>90%) of the IGFs are tightly bound to IGF-binding proteins (IGFBPs). A total of six different high-affinity binding proteins have been identified (IGFBP-1-6). Although the majority of the circulating IGFBPs are derived from the liver, many other organs also produce one or more IGFBPs (Rajaram et al. 1997, Firth & Baxter 2002, Pollak et al. 2004). Under normal circumstances, IGFBP-3 is the most abundant IGFBP in serum and binds more than 95% of the IGFs (LeRoith 1997, Firth & Baxter 2002). In normal human serum, ~70–80% of the IGFs forms a 150 kDa ternary complex with either IGFBP-3 or (to a much lesser extent) IGFBP-5, and an acidic subunit (ALS), a leucine-rich glycoprotein of ~85 kDa. Most of the residual IGFs are associated with IGFBP-1 to -6 (predominantly IGFBP-2 and -3) as smaller ~40–50 kDa binary complexes (Hardouin et al. 1989, Zapf et al. 1990, Firth & Baxter 2002). Only <1% of the IGFs circulates in the free form (Twigg et al. 1998, Bond et al. 2000, Firth & Baxter 2002). Due to its large molecular mass, the ternary complex is not able to pass the capillary membrane. Hence, the IGFs captured within this type of complex have a rather extended half-life in the circulation (T1/2 ~ 15 h) compared with the various binary complexes (T1/2 ~ 25 min) or the free unbound IGFs (T1/2 ~ 10 min). Thus, the unbound IGFs and the pool...
of IGFs associated with binary complexes in the circulation are considered to exchange relatively rapidly with the tissue compartments (Guler et al. 1989) and are more readily available for binding to IGF receptors and insulin receptors (Moller et al. 1996, Frystyk et al. 1998).

The IGF-II gene and protein

The IGF-II gene is one of the few genes known to have parental allele-specific expression. As such, it is referred to as an imprinted gene. The gene for IGF-II, together with two putative tumour suppressor genes, H19 and p57KIP2, is located on chromosome 11p15. In normal cells, the IGF-II gene is maternally imprinted in that it is expressed only from the paternal copy of the gene while H19 and p57KIP2 are expressed from the maternal allele. H19 and p57KIP2 are implicated in conserving imprinting of IGF-II (O’Dell & Day 1998, Falls et al. 1999, Khandwala et al. 2000). The IGF-II gene, together with two putative tumour suppressor genes, H19 and p57KIP2, is located on chromosome 11p15. In normal cells, the IGF-II gene is maternally imprinted in that it is expressed only from the paternal copy of the gene while H19 and p57KIP2 are expressed from the maternal allele. H19 and p57KIP2 are implicated in conserving imprinting of IGF-II (O’Dell & Day 1998, Falls et al. 1999, Khandwala et al. 2000). The IGF-II gene consists of nine exons, including six non-coding ones, with four promoters (Fig. 1). Promotor usage seems to be tissue specific and developmentally regulated which leads to multiple transcripts that all encode the same monomeric primary IGF-II translation product, pre-pro-IGF-II (Sussenbach et al. 1993). Pre-pro-IGF-II consists of 180 amino acids including a N-terminal signal peptide of the 24 amino acid residues, the 67 amino acids long mature IGF-II (7.5 kDa) and an 89 residue extension at the C-terminus. The latter has been designated the E-domain. Post-translational processing of pre-pro-IGF-II involves removal of the N-terminal signal sequence, addition of sialic acid containing oligosaccharides through O-linkage to one or more threonine residues of the E-domain, followed by sequential proteolysis of the latter extension into the mature protein. During this process a relatively stable intermediate is formed, pro-IGF-IIE (68–88), that may be secreted by the cell (Daughaday & Trivedi 1992a, Duguay et al. 1998).

Clinical features of non-islet cell tumour-induced hypoglycaemia

NICHTH is a rare paraneoplastic phenomenon. It was first described in 1929 in a patient with a hepatocellular carcinoma (Nadler & Wolfer 1929). Since then, many tumour types have been associated with hypoglycaemia. In Daughaday et al. (1988), showed for the first time that tumour-induced hypoglycaemia was associated with the aberrant production of pro-IGF-II (‘big’-IGF-II) resulting in a persistent insulin-like activity. The two new cases, we describe next, illustrate the clinical course and therapeutic problems that can be encountered in patients presenting with NICHTH.

Case reports

Case 1

A 83-year-old man was admitted with confusion and lethargy without loss of consciousness. Over the last months he had lost 8 kg in weight. His medical history revealed atrial fibrillation and epilepsy for which he

Figure 1 Structure of the insulin-like growth factor (IGF)-II gene and the precursor-IGF-II-protein. The IGF-II gene has four promoters (P1–P4). Only exons 7, 8 and a part of 9 (depicted in grey) encode for the pre-pro-IGF-II protein. The N-terminal signalling peptide (SP) is enzymatically cleaved leading to the formation of pre-pro-IGF-II. The E-domain is degraded through several steps into the mature 7.5 kDa IGF-II. Pro-IGF-II (68–88) is a relatively stable intermediary in this process.
was treated with acenocoumarol and carbamazepine. On admission, serum glucose was 1.1 mmol/l (normal fasting glucose: 4.0–5.4 mmol/l). Serum levels of insulin and C-peptide were suppressed. Sulphonylurea derivatives or insulin antibodies were not detected and the presence of a phaeochromocytoma was excluded. The concentration of serum IGF-I was reduced considerably whereas that of total IGF-II was within the normal range (molar ratio between total IGF-II and IGF-I: 17.7; reference value: <10). The serum levels of pro-IGF-IIE (68–88) (‘big’-IGF-II) were markedly elevated (Table 1). Computed tomography demonstrated a large tumour mass in the right kidney which histologically proved to be a solid fibrous tumour. No metastases were found. Serum levels of glucose, IGF-I and pro-IGF-IIE (68–88) levels normalised after nephrectomy. Histochemical analysis of tumour tissue and pro-IGF-IIE (68–88) levels are generally low or immeasurable in NICTH (Zapf 1993). As we think to be a fasting hypoglycaemia characterised by: 1) diminished hepatic glucose production due to inhibition of glycogenolysis and gluconeogenesis (Moller et al. 1991, Eastman et al. 1992, Zapf 1993); 2) diminished lipolysis in adipose tissue resulting in low serum free fatty acids levels (Zapf 1993) and 3) increased peripheral glucose consumption (Moller et al. 1991, Eastman et al. 1992, Zapf 1993, Chung & Henry 1996). These phenomena point to an enhanced insulin-like activity in the body. Furthermore, glucose consumption by the tumour itself might contribute to hypoglycaemia (Zapf 1993). As we also encountered in the present two cases, insulin levels are generally low or immeasurable in NICTH markedly elevated. She recovered quickly after administration of i.v. glucose. A carbohydrate-rich diet could hardly prevent more hypoglycaemic events and prednisolone 100 mg per day was started. Eventually, 40 mg daily was needed as maintenance therapy to prevent recurrent hypoglycaemias. Furthermore, she started with dacarbazine 800 mg/m² once every 3 weeks. After six courses, she developed lung metastases and she switched to doxorubicin 50 mg/m² every 3 weeks, which provided stable disease after six courses and, combined with steroids, kept her free of hypoglycaemic episodes for more than a year.

### Incidence and tumour types

Data on the exact incidence and prevalence of NICTH are not available. It has been estimated that NICTH is four times less common than insulinoma, but the true incidence is probably higher since many cases go unrecognised, especially concerning patients with disseminated disease (Marks & Teale 1998).

NICTH can arise in virtually every benign and malignant tumour. However, it mainly occurs in patients with solid tumours of mesenchymal and epithelial origin, but rarely also in patients with tumours of haematopoietic and neuroendocrine origin (Table 2; Zapf 1993, Frystyk et al. 1998, Marks & Teale 1998, Fukuda et al. 2006, Tsuro et al. 2006). In general, the mesenchymal tumours have in common that they are well differentiated and slowly growing, although many usually weigh between 2 and 4 kg at diagnosis.

### Symptoms

#### Hypoglycaemia

In unconscious cancer patients without signs of vascular events or brain metastases, NICTH should be considered. Subtle symptoms of hypoglycaemia, especially when they occur between meals and in the morning, can point towards the diagnosis. NICTH is thought to be a fasting hypoglycaemia characterised by: 1) diminished hepatic glucose production due to inhibition of glycogenolysis and gluconeogenesis (Moller et al. 1991, Eastman et al. 1992, Zapf 1993); 2) diminished lipolysis in adipose tissue resulting in low serum free fatty acids levels (Zapf 1993) and 3) increased peripheral glucose consumption (Moller et al. 1991, Eastman et al. 1992, Zapf 1993, Chung & Henry 1996). These phenomena point to an enhanced insulin-like activity in the body. Furthermore, glucose consumption by the tumour itself might contribute to hypoglycaemia (Zapf 1993). As we also encountered in the present two cases, insulin levels are generally low or immeasurable in NICTH

### Case 2

A 48-year-old woman was admitted in coma. She was diagnosed with haemangioepericytoma of the meninges at the age of 35 years for which she underwent surgery. She had two recurrences that were treated with radiosurgery. Five months prior to presentation a large liver metastasis was histologically confirmed. Further medical history was uneventful. On admission, serum glucose was 0.8 mmol/l and serum insulin was suppressed with normal levels of C-peptide. IGF-I, IGFBP-2 and ALS levels were below normal (Table 1). The amount of total IGF-II was within normal range. However, levels of ‘big’-IGF-II and IGFBP-2 were

### Table 1 Biochemical features of both patients with non-islet cell tumour hypoglycaemia

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<th>Patient 1</th>
<th>Patient 2</th>
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<tr>
<td>IGF-I (nmol/l)</td>
<td>3.4 (-5.5)</td>
<td>&lt;1.6 (-7.4)</td>
</tr>
<tr>
<td>Total IGF-II (nmol/l)</td>
<td>60.1 (0.9)</td>
<td>44.4 (1.4)</td>
</tr>
<tr>
<td>Pro-IGF-IIE (66–88) (nmol/l)</td>
<td>37.7 (9.3)</td>
<td>22.9 (5.4)</td>
</tr>
<tr>
<td>IGFBP2 (nmol/l)</td>
<td>ND</td>
<td>21.2 (2.6)</td>
</tr>
<tr>
<td>IGFBP3 (nmol/l)</td>
<td>ND</td>
<td>22.3 (-3.9)</td>
</tr>
<tr>
<td>Insulin (mE/l)</td>
<td>1.6</td>
<td>18</td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td>0.03</td>
<td>0.31</td>
</tr>
<tr>
<td>ALS (nmol/l)</td>
<td>ND</td>
<td>44.2 (-5.4)</td>
</tr>
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Serum levels of IGF-I, total IGF-II, pro-IGF-IIE (68–88), IGFBP-2 and IGFBP-3 were determined as described previously (Hoekman et al. 1999, van Doorn et al. 2002). Data are also expressed as SD scores for age and gender (in parenthesis). Reference ranges for insulin and C-peptide are <20 mE/l and 0.18–0.63 nmol/l respectively.
Daughaday et al. 1988, Zapf 1993, Marks & Teale 1998, Gama et al. 2003). In NICTH, the onset of symptoms is frequently gradual with lethargy, sweating, diminished motor activity and somnolence before a progressive drift into a coma. Recovery may occur spontaneously but is accelerated by the intake of carbohydrates or the administration of parenteral glucose or glucagon (Marks & Teale 1998, Gama et al. 2003).

Several studies on NICTH indicate that a major part of the glucose intake is rapidly disposed into peripheral tissues, especially skeletal muscle, rather than consumed by the tumour. Suppression of hepatic glucose production or fat oxidation does occur but seems to play a minor role in the development of hypoglycaemia (Eastman et al. 1992, Chung & Henry 1996, Zachariah et al. 2007).

Other symptoms
In addition to hypoglycaemic symptoms, acromegaloid skin changes, such as skin tags, excessive oiliness of the skin and rhinophyma, have been described in patients with NICTH (Trivedi et al. 1995, Bertherat et al. 2000). Elevated serum levels of total IGF-II are frequently found in acromegalic patients and it is known that prolonged activation of the IGF1R by IGF-II may contribute to the development of acromegaloid features (LeRoith et al. 1995, Renehan et al. 2001). Therefore, it is conceivable that the secretion of high molecular weight forms of IGF-II by tumour tissue into the circulation might play a role in the development of external signs of acromegaly in some NICTH patients.

Clinical course
NICTH can be either the presenting symptom of a tumour or present in patients with a history of a neoplasm. In a large study from Japan, describing 78 patients, the clinical course of patients with NICTH was analysed (Fukuda et al. 2006). In 48% of these cases, a hypoglycaemic episode was the initial sign that led to the diagnosis and the discovery of a tumour. In the remainder of the patients, hypoglycaemia was detected during the period of observation and treatment of the underlying malignancy. Furthermore, 13% of the NICTH patients investigated had a histologically identical tumour resected in the past without any evidence of hypoglycaemia at that time. Vice versa, and quite surprisingly, hypoglycaemia does not always reappear when a previously NICTH-causing tumour recurs and grows back to its former size.

(Pro-) IGF-II is expressed in a broad spectrum of malignant (and benign) tumours and IGF-II may act as...
an autocrine growth factor through binding to the IGF1R or the isoform A of the insulin receptor which conduct a strong mitogenic and antiapoptotic activity (Baserga et al. 2003, LeRoith & Roberts 2003). Although both IGF-II and ‘big’-IGF-II are capable of inducing the phosphorylation of partially purified preparations of both the insulin receptor and IGF1R (Zapf et al. 1981, Hoekman et al. 1994), it has not been unambiguously demonstrated that ‘big’-IGF-II indeed stimulates tumour progression (Duguay et al. 1998). Furthermore, it is not known whether the existence of NICHT is of prognostic value with respect to predicting the degree of malignancy of a tumour or the (disease-free) survival of the patient.

Pathogenesis of non-islet cell tumour hypoglycaemia

Involvement of IGFs

The incongruity between the clear insulin deficiency in patients with NICHT on the one hand and metabolic features pointing to enhanced insulin action on the other hand suggests that IGFs play a major role in NICHT. In contrast to insulin and IGF-I which are under endocrine control, IGF-II production is predominantly autocrine and paracrine. The level of IGF-I in serum of NICHT patients is usually decreased. On the other hand, circulating levels of total IGF-II, as determined by conventional immunometric or receptor assays, may be either increased, decreased or within the normal range (Zapf et al. 1981, Hoekman et al. 1999, van Doorn et al. 2002).

These puzzling observations were clarified by Daughaday et al. (1988). The concentration of total IGF-II levels in serum from a NICHT patient with a leiomyosarcoma investigated by them was within the normative range as measured by both RIA and radioreceptorassay. However, when the patient’s serum was subjected to Biogel P-60 column chromatography at acidic pH, about 70% of the total IGF-II appeared to be recovered in a higher molecular weight (10–17 kDa) fraction. The remainder of the IGF-II was in the mature 7.5 kDa form. In contrast, in normal serum, high molecular forms of IGF-II (‘big’-IGF-II) contributed only in about 10–20% to the total IGF-II pool. Furthermore, the tumour contained high concentrations of IGF-II mRNA. The authors also demonstrated that after removal of the tumour, ‘big’-IGF-II levels in the patient’s serum normalised (Daughaday et al. 1988). These findings suggested that the IGF system indeed was involved in pathogenesis of NICHT.

Aberrant IGF expression in NICHT

As illustrated in the case of patient 1, NICHT-causing tumours abundantly express IGF-II mRNA. IGF-II gene overexpression is more widespread than first thought and appears to occur in various malignancies, albeit to a variable extent (Hodzic et al. 1997, van der Ven et al. 1997). Nowadays it is generally accepted that IGF-II is involved in oncogene-induced tumorigenesis (Khandwala et al. 2000, Samani et al. 2007). Overexpression of IGF-II in tumours has been mainly attributed to either a loss of imprinting or mutations in tumour suppressor genes (Drummond et al. 1992, Christofori et al. 1995, Khandwala et al. 2000). The mechanisms leading to IGF-II mRNA overexpression in NICHT have rarely been studied (Hodzic et al. 1997, Bertherat et al. 2000). Hodzic et al. (1997) reported loss of imprinting of the IGF-II gene in a mesothelioma causing hypoglycaemia. In addition, Bertherat et al. (2000) studied allele-specific expression of the IGF-II gene in a pleural fibrosarcoma causing NICHT. They observed a loss of imprinting of both parental alleles causing increased expression of the IGF-II gene and decreased expression of the genes encoding the tumour suppressors H19 and p57KIP2.

Besides elevated levels of mRNA for IGF-II, in some cases tumours causing NICHT occasionally also express mRNAs for either IGFBP-4, -5- or -6 (Baxter et al. 1995, Holt et al. 1998, Hoekman et al. 1999, Silveira et al. 2002). Patients with NICHT usually exhibit elevated levels of IGFBP-2 in their circulation but the source (with exception of one reported case with a IGFBP-2 expressing tumour; Silveira et al. 2002) and pathophysiologic role of this IGFBP are not established (Baxter et al. 1995, Holt et al. 1998, Hoekman et al. 1999, Silveira et al. 2002).

‘Big’-IGF-II and NICHT

Not all tumours that overexpress the IGF-II gene cause NICHT and many NICHT cases involve pre-existing tumours. It is not clear whether serum pro-IGF-II levels in patients with these tumours are already elevated prior to the first signs of hypoglycaemia. In non-islet cell tumours causing hypoglycaemia, post-translational processing of pro-IGF-II is abnormal (Daughaday 1990, Shapiro et al. 1990, Zapf et al. 1992, Hizuka et al. 1998). As depicted in Fig. 1, overexpression of the IGF-II gene causes overproduction of pro-IGF-II (Daughaday 1990). Incompletely processed pro-IGF-II accounts for 10–20% of the total IGF-II in the normal human serum (Daughaday & Trivedi 1992b) and is O-glycosylated (Hudgins et al. 1992, Daughaday et al. 1993). In serum of patients with NICHT, a much higher
proportion (usually >60%) of IGF-II is in a higher molecular weight form that seems to be non-glycosylated and consists primarily of IGF-II with a 21 amino acid extension of the E-domain (pro-IGF-IIE (68–88); Daughaday et al. 1988, 1993, Zapf et al. 1992, Kuenen et al. 1996). Glycosylation may therefore be a targeting signal for cleavage of the E-domain peptide and contribute to the size heterogeneity observed in ‘big’-IGF-II. It seems likely that in many neoplastic cells the levels of the various enzymes involved in post-translational processing are not sufficient to handle the relatively high amounts of pro-IGF-II produced adequately (Megyesi et al. 1974, Gorden et al. 1981, Axelrod & Ron 1988, Daughaday et al. 1988, Lowe et al. 1989, Ron et al. 1989, Shapiro et al. 1990, Teale & Marks 1990, Daughaday & Trivedi 1993, Zapf 1993, Marks & Teale 1998, van Doorn et al. 2002).

As emphasised previously, ‘big’-IGF-II is biologically active and is present in relatively high amounts in the serum of NICTH patients. In most cases, the serum levels of total IGF-II are not elevated. Therefore, it seems that ‘big’-IGF-II must have specific biochemical properties, being different from those of mature IGF-II that lead to an enhanced bioavailability and, consequently, increased insulin-like activity in the body (Zapf et al. 1992).

‘Big’-IGF-II has equal affinity for the IGFBPs compared with fully processed IGF-II and can therefore form the binary complex with all IGFBPs (Zapf et al. 1992, Bond et al. 2000). However, although the exact mechanism is still unknown, ‘big’-IGF-II seems to possess properties, which do not allow the proper formation of a 150 kDa complex together with IGFBP-3 and ALS (Fig. 3). It seems that in NICTH the binary complex of ‘big’-IGF-II and IGFBP-3 has a strongly reduced affinity for ALS since a deficiency in or dysfunction of ALS do not occur (Baxter & Daughaday 1991, Daughaday et al. 1995, Daughaday 2004).

Possibly, the heavy N-linked carbohydrate moiety of IGFBP-3, which is absent from IGFBP-5, may interact with the E-domain of ‘big’-IGF-II leading to steric interference and consequently reduction of the affinity for ALS (Bond et al. 2000, Daughaday 2004). Indeed, IGFBP-5 is still capable of forming ternary complexes with IGF-I and ALS (Bond et al. 2000). As a consequence of impaired formation of the 150 kDa complex, tumour-derived ‘big’-IGF-II primarily forms smaller binary complexes with IGFBPs and a greater fraction may stay in the free unbound form (Daughaday & Kapadia 1989, Zapf et al. 1990, 1992). These smaller complexes have a greater capillary permeability and thus are thought to increase IGF bioavailability to the tissues, resulting in hypoglycaemia through action on the insulin receptors and IGFR1. In the light of this, it is fascinating that tumours causing NICTH display elevated serum levels of particular IGFBPs (Baxter et al. 1995, Holt et al. 1998, Hoekman et al. 1999, Silveira et al. 2002). Apparently, some tumours can be more or less self sufficient in delivering IGF-II from the circulation to its target tissues causing hypoglycaemia.

Figure 3 Analysis of complex formation in patient 2 using S200 column chromatography. An aliquot of patient’s serum (250 μl) or normal control serum was incubated with 50 μl [125I]-labelled purified human insulin-like growth factor (IGF)-I (100 000 c.p.m.) and the different complexes were subsequently separated. In patient’s serum, 150 kDa complex formation is reduced and, instead, relatively higher proportions of [125I]-IGF-I are associated with the smaller 40–50 kDa binary complexes and remain unbound.
Thus, as summarised in Fig. 4, it can be hypothesised that excessive production of 'big’-IGF-II by a tumour leads in fact to a vicious circle whereby the impaired formation of ternary complexes is gradually worsened by an increasing feedback inhibition of GH production that reduces the amounts of IGFBP-3 and ALS available for complex formation further. Quite probably, hypoglycaemia occurs when the various counter-regulatory processes cannot compensate anymore for the increasing insulin-like activity.

Other causes of hypoglycaemia

Of course, other combinations of factors may occur as well. Decreased hepatic glucose output associated with destruction of the liver by tumour infiltration may also be a critical factor in the onset of hypoglycaemia. In addition, sometimes patients with NICTH do not have elevated 'big’-IGF-II levels and hypoglycaemia may be caused by the increased secretion of mature instead of 'big’-IGF-II (Zapf 1993), IGF-I (Nauck et al. 2007),

**Figure 4** Proposed mechanism of the development of non-islet cell tumour hypoglycaemia (NICTH). It is likely that tumour cells cannot process the augmented amounts of pro-insulin-like growth factor-II (pro-IGF-II) synthesised, resulting in a substantial release of 'big’-IGF-II into the circulation. 'Big’-IGF-II competes with the mature IGF-II and IGF-I for binding to IGF-binding proteins (IGFBPs). However, the formation of the ternary complex between 'big’-IGF-II, IGFBP-3 and the acid-labile subunit (ALS) in the circulation is hampered. As a consequence, primarily 40–50 kDa binary complexes are formed. In addition, both free fractions of IGF-I and total IGF-II are increased. Since the binary complexes and free IGFs can pass the capillary membrane relatively easily when compared with the 150 kDa ternary complex, the concentrations of IGFs, presumably especially 'big’-IGF-II, at the tissue level will rise inducing a strong insulin-like effect via the insulin receptors, causing hypoglycaemia. Moreover, because of an increasing negative feedback on growth hormone (GH) production by the anterior pituitary gland the synthesis of GH-dependent peptides such as IGF-I, IGFBP-3, IGFBP-5 and acid-labile subunit (ALS) are decreased. This leads to even further declined formation of ternary complexes.

Abbreviations: FFA, free fatty acids.
insulin or other peptides with insulin-like activity (Marks & Teale 1998, Todd et al. 2003) or a combination of cachexia, renal and hepatic dysfunction, and glucose consumption by the tumour (Singh et al. 2006).

‘Big’-IGF-II and other disease entities

There are other disease entities in which abnormal processing of pro-IGF-II plays a role in the aetiology and/or pathophysiology. For example, Daughaday et al. (1990) reported that individuals who have immunologic markers of hepatitis B virus infection may exhibit an increased proportion of partially processed pro-IGF-II in their circulation. However, these patients had no evidence of hypoglycaemia.

Patients with hepatitis C-associated osteosclerosis (HCAO) have a specific increase in circulating ‘big’-IGF-II and IGFBP-2 levels. However, HCAO patients do not exhibit hypoglycaemia, nor have NICTH patients been reported to have osteosclerosis. The predominant circulating forms of ‘big’-IGF-II in HCAO and NICTH are clearly different, including pro-IGF-IIIE (1–104) and pro-IGF-IIIE (1–88) respectively perhaps accounting for the development of osteosclerosis in one syndrome and hypoglycaemia in the other (Khosla et al. 2002). Furthermore, in HCAO the 150 kDa ternary complexes in serum are formed normally and there is no increase in serum free IGFs (Khosla et al. 1998).

Diagnosis

NICTH suppresses insulin secretion by beta-cells, lipolysis and ketogenesis (Moller et al. 1991, Eastman et al. 1992, Zapf 1993), leading to low C-peptide, and inappropriately low GH and β-hydroxybutyrate (β-OH(B) concentrations in the circulation (Marks & Teale 1998, Gama et al. 2003). In case of hypoinsulinaemic hypoglycaemia, the assessment of elevated serum levels of ‘big’-IGF-II or E (68–88)-peptide, in combination with increased levels of IGFBP-2 by specific immunometric assays is of high diagnostic value (van Doorn et al. 2002). Size-exclusion acid chromatography, a very time-consuming procedure, has been considered the gold standard method for detection of ‘big’-IGF-II in NICTH. However, measurement of the serum concentration of ‘big’-IGF-II determined by immunoblot analysis of ‘big’-IGF-II and mature IGF-II after 16.5% tricine-sodium dodecyl sulphate–polyacrylamide gels has proven to be a more rapid, reproducible and equally sensitive method and a useful laboratory evaluation of patients with a clinical diagnosis of NICTH (Miraki-Moud et al. 2005).

Since GH secretion is restrained with subsequent lowering of GH-dependent IGF-I and IGFBP-3 production by the liver (Fig. 4), reduced levels of the latter proteins in serum represent useful additional markers, as well as an increased ratio between total IGF-II and IGF-I. Despite hypoglycaemia, levels of glucagon are often within the normal range suggesting a suppressive effect of ‘big’-IGF-II on glucagon secretion (Chung & Henry 1996, Fehmann et al. 1996). However, in the absence of diffuse liver metastases, tumour-induced hypoglycaemia often is associated with increased, rather than depleted, hepatic glycogen stores (Phillips & Robertson 1993, Chung & Henry 1996). Furthermore, hypokalaemia is often associated with hypoglycaemia, presumably due to the insulin-like activity of (‘big’-) IGF-II (Fukuda et al. 2006).

Since, tumours causing NICTH are usually very large, they can be readily detected by conventional radiological imaging using computed tomography or magnetic resonance imaging. However, one has to bear in mind that functional tumour imaging techniques such as fluorodeoxyglucose-positron emission tomography may lead to false-negative results (de Boer et al. 2006). Presumably this is due to an accelerated uptake of fluorodeoxyglucose by especially the heart and skeletal muscle that competes with the slower rate of uptake of tracer by tumour tissue. Other nuclear tracers such as radiolabelled tyrosine can provide an alternative (Jager et al. 2001).

Treatment

Curative and palliative measures

The long-term therapeutic strategies in NICTH involve complete removal of the tumour or reduction of the tumour mass. The metabolic alterations caused by NICTH are fully reversible after successful surgical removal of the ‘big’-IGF-II producing tumour (Daughaday et al. 1988, Zapf et al. 1992, Zapf 1993), as also demonstrated in case 1.

In many cases, including patient 2, the malignancy causing NICTH is a large mass infiltrating into surrounding tissue and often accompanied by disseminated disease. Alleviating hypoglycaemia is subsequently a therapeutic challenge. When curative resection is no longer possible, various approaches to the treatment of NICTH have been tried with the initial aim of relieving the hypoglycaemic symptoms. Chemotherapy directed against the tumour or selective
embolisation of tumour mass can also reduce the occurrence of hypoglycaemic events. However, when the tumour has only partially disappeared, hypoglycaemia is likely to reoccur when the tumour grows again (Nanayakkara et al. 2002).

**Increasing serum glucose**

In order to treat hypoglycaemia, a short-term beneficial effect is best achieved with (continuous) parenteral administration of glucose and dietary guidelines. However, especially when taken into account the condition of the patient, these measures are sometimes difficult to realise. As in insulinomas, diazoxide–chlorothiazide treatment may improve NICTH symptoms (Marks & Teale 1998). Correction of hypoglycaemia also has been attempted successfully by the administration of glucagon. It does so primarily by increasing hepatic glucose output (Phillips & Robertson 1993). Interestingly, Hoff & Vassilopoulou-Sellin (1998) reported that a glycaemic response to a glucagon stimulation test predicted good response to long-term treatment with glucagon (0.06–0.3 mg/h), via continuous i.v. infusion.

**Somatostatin analogues**

The presence of somatostatin receptors has been demonstrated previously in a pleural fibroma causing NICTH (Perros et al. 1996) and in ~40–55% of hepatocellular carcinomas (Reubi et al. 1999, Cebon 2006). However, in NICTH the administration of somatostatin analogues such as octreotide, generally does not restore glucose levels, probably because somatostatin receptors, if present at all in the hypoglycaemia causing tumour, are non-functional (Hunter et al. 1994, Perros et al. 1996, Morbois-Trabut et al. 2004). Nonetheless, in a case of an intra-abdominal haemangiopericytoma, the prolonged infusion of somatostatin appeared to reduce the secretion of ‘big’-IGF-II by the tumour (Chung & Henry 1996).

**Glucocorticosteroids**

Glucocorticosteroid treatment seems to be the most effective one in terms of long-term relief from hypoglycaemia by stimulating gluconeogenesis and suppressing, although not in all cases, the production of ‘big’-IGF-II and correcting the attendant biochemical abnormalities involving the GH–IGF axis (Baxter et al. 1995, Perros et al. 1996, Teale & Marks 1998, Bourcigaux et al. 2005). Moderate to high doses of glucocorticosteroids may cause shrinkage of the tumour. A recent study extended these findings by demonstrating that the beneficial effects of glucocorticosteroids are dose dependent and reversible when treatment is withdrawn or when the dose falls below a critical level (Teale & Wark 2004).

**Growth hormone**

In a recent review (Holt et al. 2003), the stimulation of IGFBP-3 and ALS production by the liver after the administration of rhGH was considered to be beneficial in the treatment of NICTH. However, in two cases of NICTH associated with pleural solitary fibrous tumours, successful GH treatment lead to only moderate increments of circulating IGF-I and IGFBP-3 levels (Drake et al. 1998). This would suggest that alternative, possible direct, mechanisms of action of GH in alleviating hypoglycaemia most likely play a role. Although increases in serum concentrations of IGFBP-3 and ALS indisputably occur, it seems that either the production rise is insufficient and/or the amount of tumour derived ‘big’-IGF-II is still sufficient to inhibit formation of ternary complexes (Silveira et al. 2002, Bourcigaux et al. 2005). Analogous to the induction of glucose intolerance in acromegaly, stimulation of hepatic gluconeogenesis and glycogenolysis may be an important aspect of the effect of (recombinant) GH. As demonstrated previously (Drake et al. 1998, Teale & Marks 1998), GH can alleviate hypoglycaemia. Aside from their influence on serum insulin and glucose, the diverse metabolic effects of GH (in protein sparing) and glucocorticosteroids (in tumour suppression) suggest that their combined use may be feasible in the treatment of NICTH (Horber et al. 1991, Baxter et al. 1995, Teale & Wark 2004, Bourcigaux et al. 2005).

**Conclusion**

In patients with a mesenchymal or malignant epithelial tumour suffering from hypoglycaemic episodes or unconsciousness, NICTH should be considered. NICTH follows the production of partially processed forms of pro-IGF-II, called ‘big’-IGF-II, which, apart from direct insulin-like biological activity, fails to form inactive ternary complexes with IGFBPs and ALS and would increase bioavailability of IGFs. At the target tissues (‘big’), IGF-II interacts with IGF1R and insulin receptors resulting in hypoglycaemia. Low serum insulin in combination with elevated levels of ‘big’-IGF-II and an increased IGF-II:IGF-I ratio would confirm the diagnosis. Removal of the tumour can cure NICTH but when that is no longer possible, treatment
with glucocorticosteroids, GH or combinations thereof can suppress NICTH and alleviate symptoms.

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