MANAGEMENT OF ENDOCRINE DISEASE

Etiology and outcome of acromegaly in patients with a paradoxical GH response to glucose

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Abstract

To gain more insight on the pathogenesis of somatotroph pituitary adenomas, recent studies have focused on a subgroup of patients with acromegaly displaying a paradoxical growth hormone (GH) response during oral glucose tolerance test (OGTT). The paradoxical rise of GH after oral glucose intake occurs in about one-third of acromegaly patients and has been pathogenetically linked, by analogy to glucose-dependent insulinotropic polypeptide (GIP)-dependent Cushing's syndrome, to the ectopic expression of GIP receptor (GIPR) in somatotroph adenoma cells. GIPR-expressing adenomas are negative for activating *GNAS* gene mutations and display distinct cytogenetic and DNA methylation profiles, highlighting their unique molecular pathogenesis. Acromegaly patients with a paradoxical GH response pattern seem to display higher insulin-like growth factor-1 (IGF-1) concentrations and harbour smaller adenomas that are more often of the densely granulated phenotype. They seem also to show a better response to somatostatin receptor ligands. In addition, persistent paradoxical GH response after surgery may be a biological marker of the residual disease postoperatively. Targeted therapy to antagonize GIP receptor on GIPR-expressing somatotroph adenomas could be a new treatment approach for acromegaly patients with a paradoxical pattern of GH response to OGTT.

*European Journal of
Endocrinology*
(2021) **184**, R261–R268

Invited Author's profile

Peter Kamenický is full professor of endocrinology at the Bicêtre Hospital and Paris-Saclay University, France. Since 2020, he is also director of the Inserm / Paris-Saclay University research unit UMR-S 1185, conducting translational research in several fields of endocrine physiology and pathophysiology. Professor Kamenický's research has mainly focused on the pathophysiology of pituitary diseases such as acromegaly and Cushing's syndrome and pituitary and adrenal tumorigenesis. In parallel, he has developed an extensive clinical experience in the management of diseases of calcium and phosphate metabolism, such as X-linked hypophosphatemia and hypoparathyroidism in adults.

Introduction

Somatotroph pituitary adenomas are responsible for chronic GH hypersecretion resulting in gigantism during childhood and acromegaly during adulthood. Despite recent advances in the understanding of the molecular pathogenesis of somatotroph adenomas, the driver events promoting somatotroph adenoma development remain poorly understood. Activating somatic mutations in *GNAS* gene, which encodes the G protein stimulatory alpha subunit, are identified in around 30% of somatotroph adenomas (1). Furthermore, somatotroph adenomas may occur in association with several very rare genetic predisposition syndromes, linked to mutations in *AIP*, *MEN1*, *CDKN1B* and *PRKAR1A* genes (2,3,4,5,6,7,8).

Since several studies based on next generation sequencing technologies have failed to identify novel genetic events involved in somatotroph tumorigenesis (9,10,11,12), alternative mechanisms have been considered. In particular, over the last years, attention has been focused on a specific group of patients with acromegaly presenting with a paradoxical increase of serum GH concentrations after oral glucose load (13,14,15). Similar to GIP-dependent Cushing's syndrome (16, 17), the paradoxical rise in GH concentrations has been linked to the ectopic expression of the glucose-dependent insulinotropic polypeptide (GIP) receptor (GIPR) in somatotroph adenomas.

Around one-third of somatotroph adenomas express ectopically GIPR (13, 15). This subgroup of somatotroph adenomas, biochemically identified by a paradoxical increase of GH during an oral glucose tolerance test (OGTT), are further characterized by specific molecular profiles (14, 15) and different clinical outcomes, as recently highlighted (18). In this review, we will present the known mechanisms underlying the paradoxical GH rise during OGTT and its significance in the clinical presentation and outcome of patients with acromegaly and GIPR-expressing adenomas.

Methodology for literature search

A PubMed search was conducted from the period of 1946 until 2020 using the search terms: 'acromegaly', 'ectopic expression', 'glucose-dependent insulinotropic polypeptide', 'glucose-dependent insulinotropic polypeptide receptor', 'growth hormone', 'oral glucose tolerance test', 'paradoxical response', 'somatostatin analogues', 'somatotroph pituitary adenomas'.

The above terms were used in mixed combinations. Boolean operators and truncations were used to expand our search results. References from the selected pertinent articles, and publications available in the authors' libraries were also used.

Definition

GH suppression during OGTT along with IGF-1 measurements constitute the basis of the biochemical diagnosis of acromegaly (19). Failure to suppress GH levels below 0.4 µg/L after a 75 g oral glucose load is a hallmark of autonomous GH secretion (20), and this cut-off value when using current ultrasensitive GH assays should be probably even lower (21). The influence of glucose on GH secretion has been shown since the early 1960s (22). However, to date, the precise mechanisms of GH suppression by oral glucose are not completely understood and are hindered by species disparities in the physiological response of GH to glucose overload as previously reviewed in detail (23). GH suppression seems to be most likely related to a glucose mediated increase in somatostatin release (24, 25). In healthy individuals, GH secretion in response to GH releasing hormone (GHRH) or GH secretagogues is reduced after an oral glucose load. In addition, the acetylcholinesterase inhibitor pyridostigmine which suppresses somatostatin release reverses the inhibitory effect of glucose on GH (26).

In around one-third of patients with acromegaly, GH levels can paradoxically increase in response to oral glucose as shown in Fig. 1 (23). There is no current agreement on the criteria to define a 'paradoxical pattern' of GH response to oral glucose load. Different studies have used different

Figure 1

Patterns of GH response during OGTT in patients with acromegaly. (A) A classical response of GH during OGTT where GH concentrations decrease but are not suppressed below the cut-off value of 0.4 µg/L. (B) A paradoxical GH response during OGTT, with an early GH rise in response to oral glucose load.

Review	M Hage and others	Paradoxical GH response pattern in acromegaly	184:6	R263
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Table 1 Studies of paradoxical GH response to OGTT in patients with pituitary adenomas.

References	Number of patients	Glucose load	Definition of paradoxical response	Prevalence of paradoxical response (%)
(28)	22 pituitary adenomas	100 g	↑ GH	2 acromegaly (9%); 1 optic glioma
(30)	37 controls ; 16 acromegaly	75 g	>50% from baseline level at 30–60 min	3 controls (8%)
(31)	22 acromegaly	100 g	↑ GH	2 acromegaly (9%)
(27)	19 acromegaly	NA	↑ GH	3 acromegaly (16%)
(29)	145 acromegaly	NA	↑ GH	38 acromegaly (26%)
(13)	21 acromegaly	75 g	↑ GH (61% ± 24%) within 30–90 min; ↑ GH > 90 min in one patient	7 acromegaly (33%)
(18)	496 acromegaly	75 g	Peak to basal GH ratio > 120%	184 acromegaly (37%)
(32)	64 acromegaly	75 g	↑ GH by 30% from baseline	19 acromegaly (30%)
(15)	41 acromegaly	75 g	↑ GH by 50% from baseline within first 90 min	10 acromegaly (24%)

GH, growth hormone; min, minute; OGTT, oral glucose tolerance test.

cut-offs to define a paradoxical GH response varying from a non-defined early increase in GH to an increase ranging from 20 to 100% in GH levels as summarized in Table 1 (13, 15, 18, 27, 28, 29, 30, 31, 32). In our recent study, we defined a paradoxical GH increase to oral glucose by a 50% rise in GH within the first 90 min (15). This cut-off takes into account the well-documented fluctuations in spontaneous GH secretion in patients with acromegaly (33) and allows to distinguish the early rise in GH concentrations from the late GH rebound after initial suppression.

Of note, data on the reproducibility of GH response pattern to OGTT is lacking and further studies are needed to refine the definition of paradoxical pattern of GH response to oral glucose load.

Pathogenesis

Although the phenomenon of paradoxical GH response in acromegaly has been described since 1966 by Beck *et al.* (28), little has been known about its pathogenesis until recently. Earlier studies suggested that it is a result of an altered interplay between somatostatin and GHRH (34). Recently, the link between the ectopic expression of GIPR in somatotroph adenomas and the presence of a paradoxical GH rise to oral glucose has been evoked (13, 35) by analogy to GIP-dependent Cushing's syndrome (16, 17). In patients with GIP-dependent Cushing's syndrome, postprandial GIP release activates aberrantly expressed

adrenal GIPRs, functionally coupled to cAMP signalling, resulting in excessive cortisol secretion. Accordingly, activation of GIPR aberrantly expressed in somatotroph cells results in an increase in serum GH concentrations in response to GIP, secreted from intestinal K-cells after a stimulus such as oral glucose or food intake (Fig. 2). Initial studies by Umahara *et al.* showed that oral glucose but not i.v. glucose load increased plasma GH levels in two patients with acromegaly (35). Furthermore, i.v. GIP infusion increased GH concentrations in these two patients. *In vitro*, in GH3 cells transfected with GIPR, GIP stimulation increased cAMP levels and GH gene transcription (13). Stimulation with GIP also increased GH release in a proportion of GIPR-expressing somatotroph adenomas in primary cultures (14).

All the previously mentioned data suggest a causal role of pituitary GIPR in the pathogenesis of paradoxical GH response pattern. We have recently confirmed this link by showing that among 41 somatotroph adenomas, all 10 samples from patients with paradoxical GH responses to oral glucose displayed ectopic GIPR expression (15).

Of note, none of the GIPR-expressing somatotroph adenomas in our study harboured any *GNAS* mutation confirming observations by Occhi *et al.* (13). This suggests that the presence of activating *GNAS* mutations and the expression of GIPR in somatotroph adenoma cells, both resulting in enhanced cAMP signaling, are mutually exclusive. Interestingly, we found greater levels of *GHRHR* transcripts in GIPR-expressing adenomas. *GHRHR* is a

Figure 2
Links between GH secretion and ectopic GIPR expression in somatotroph adenoma cells. GIP release from intestinal K cells induced by meal or oral glucose during OGTT activates aberrantly expressed GIPR on somatotroph adenoma cell. GIPR is functionally coupled to cAMP signalling, similarly to the GHRH receptor. Activation of the ectopic GIPR results in an increase of cAMP signalling which stimulates GH secretion through transcriptional and likely posttranscriptional mechanisms. The molecular hits (yellow star) underlying the ectopic GIPR expression include cytogenetic alterations and epigenetic dysregulation of *GIPR* locus and probably other unknown events.

gene regulated by cAMP signalling. Hence, its expression is induced by the activation of GIPR on somatotroph cells as depicted in Fig. 2 (36).

The molecular mechanisms behind the ectopic expression of GIPR are not well elucidated. We have recently demonstrated that duplications in chromosome region 19q13.32 containing the *GIPR* gene are responsible for ectopic *GIPR* expression in two adrenocortical adenomas with GIP-dependent Cushing's syndrome. These microduplications are associated with chromosomal rearrangements creating a novel genomic environment that permits *GIPR* transcription in adrenal lesions (37). Thus, we searched for similar mechanisms that could explain the ectopic *GIPR* expression in somatotroph adenomas (15). By RNA fluorescence *in situ* hybridization, we showed that *GIPR* expression occurred through transcriptional activation of a single allele of the *GIPR* gene in the majority of GIPR-expressing somatotroph adenoma samples. We further detected microamplifications of *GIPR* locus in some GIPR-expressing adenomas that could account for ectopic *GIPR* expression. In addition, we found that GIPR-expressing adenomas displayed a global DNA hypermethylation when compared to *GNAS* mutated adenomas not expressing *GIPR* and in particular hypermethylation in the *GIPR* gene body. Since gene body DNA methylation has been shown to

enhance transcriptional efficiency (38, 39), this mechanism may contribute to ectopic GIPR in the pituitary.

Clinical and histological features

We observed that patients with GIPR-expressing adenomas were more often males and similarly there

Table 2 Characteristics of patients with acromegaly and paradoxical GH response.

Characteristics	References
Clinical features	
Male predominance	(15), (14)
Older patients	(18)
Better response to somatostatin receptor ligands	(18)
Biological characteristics	
Higher levels of IGF-1	(15)
Lower likelihood of prolactin co-secretion	(15), (18)
Adenoma characteristics	
Smaller and less-invasive adenomas	(18)
More often densely granulated	(15), (18)
GIPR receptor expression	(15), (14)
Absence of <i>GNAS</i> mutation	(15), (14)
High cytogenetic burden	(15)
DNA hypermethylation	(15)

GH, growth hormone; GIPR, glucose-dependent insulinotropic polypeptide receptor; IGF-1, insulin-like growth factor 1.

was a male predominance in patients with a paradoxical GH response although the latter was not statistically significant (15). Patients with paradoxical response also had higher IGF1 levels normalized to age and sex. We found no difference in age at diagnosis, remission rates, size or invasive behaviour of the tumours between patients with paradoxical and classical GH response. In an Italian series of 47 somatotroph adenomas, Regazzo *et al.* found a trend towards a higher prevalence of male gender and higher serum GH levels among patients with GIPR-expressing adenomas. There was no difference in age at diagnosis, basal plasma prolactin level, and adenoma size (14). Since the expression of GIPR is not synonymous with a paradoxical GH response

pattern, additional analysis would be needed to confirm these findings in patients with paradoxical GH responses, even though the two groups showed considerable overlap (paradoxical GH increase in 80% of GIPR-expressing adenomas). In a recent large retrospective clinical study involving 496 patients with acromegaly, a paradoxical response was observed in 181 (37%) patients (18). Patients with paradoxical GH response were older but no difference in gender was reported. Higher levels of IGF-1 were observed in the group of patients with a paradoxical GH response in addition to a lower likelihood of hyperprolactinemia, probably due to less prevalent stalk effect. Indeed, patients with a paradoxical GH response had smaller and less invasive tumours (18).

Figure 3

GH responses during OGTT in two acromegaly patients preoperatively and following transphenoidal surgery. GH responses during OGTT are represented as per cent of the baseline concentration. T1-weighted coronal MRI after gadolinium injection and T2-weighted coronal MRI before surgery (blue boxes) and 2 years after surgery (red boxes) are shown. (A) Abolishment of paradoxical GH response after transphenoidal surgery in a patient with complete biological remission and no residual tumour visible on MRI. (B) Persistence of paradoxical GH response in a patient with small residual tumour and normal IGF-1 concentrations under cabergoline treatment.

Histologically, all adenoma derived from patients with paradoxical GH responses and all GIPR-expressing adenomas in our study were 'pure' somatotropinomas, the majority of which was of the densely granulated phenotype (15). Similarly, Scaroni *et al.* in their study found a trend towards more densely granulated phenotype among acromegaly patients with paradoxical GH response (18). Furthermore, the majority of the tumours from patients with paradoxical GH responses in our study displayed a high copy number alteration profile on array-CGH analysis highlighting their unique molecular pathogenesis (40). The characteristics of patients with a paradoxical GH response and GIPR-expressing adenomas are summarized in Table 2.

Treatment outcomes and prognostic value

The presence of a paradoxical GH response seems to be a marker of treatment responsiveness to somatostatin receptor ligands (SRLs). Scaroni *et al.* demonstrated a better response to SRLs in patients with a paradoxical GH response compared to patients with a classical GH response to OGTT (76% vs 50%, $P = 0.001$). This observation is not surprising as the majority of GIPR-expressing adenomas are densely granulated adenomas and the latter were shown to display better responses to SRLs (41, 42). In the future, a paradoxical pattern of GH response to oral glucose may help to identify GIPR-expressing adenomas potentially responsive to GIP antagonist GIP(3-30)NH(2), a novel treatment reported to successfully antagonize GIP actions in human studies (43).

Finally, the persistence of a paradoxical GH response to OGTT in patients with acromegaly after surgical treatment could help to recognize patients with residual disease early on post-operatively (Fig. 3) before expected decrease of IGF-1 levels or in the case of microscopic residual disease difficult to detect by MRI. This prognostic value could be comparable with that of desmopressin test in Cushing's disease after surgical treatment (44). Disappearance of a response to desmopressin after surgery may suggest complete removal of adenomatous corticotrophs cells and a lower possibility of recurrence. Hence, it would be interesting to study the evolution of a paradoxical GH response in a large cohort of patients after pituitary surgery and its relationship with residual disease.

Conclusion

A paradoxical GH increase during OGTT can be seen in around one-third of patients with acromegaly, even though the definition of the paradoxical response pattern

needs to be refined. This paradoxical rise of GH after oral glucose intake is pathogenetically linked to ectopic GIPR expression in somatotroph adenomas that are negative for activating *GNAS* mutations. Acromegaly patients with paradoxical GH response seem to have higher IGF-1 concentrations and smaller tumours that are more often of densely granulated phenotype. Paradoxical response seems to be also associated with better response to somatostatin receptor ligands and may be a marker of persistence disease after surgery. Further work is needed to assess the functional involvement of ectopic GIPR activation in secretory properties of somatotropinomas as well as the usefulness of OGTT in the post-operative follow-up of these patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by Agence National de la Recherche (ANR INTE-GRAL 18-CE14-0021). JC received a fellowship from Inserm (Plan Cancer - Soutien pour la Formation à la Recherche Translationnelle en cancérologie).

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Review	M Hage and others	Paradoxical GH response pattern in acromegaly	184:6	R268
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Received 17 December 2020

Revised version received 25 March 2021

Accepted 07 April 2021