



The usefulness of the GHRH stimulation test in the diagnostics of growth hormone deficiency in children

Przydatność testu GHRH w diagnostyce niedoboru hormonu wzrostu u dzieci

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Abstract

Introduction: Stimulation tests form the basis for the diagnostic process in growth hormone deficiency (GHD). One of these tests uses the GH releasing hormone (GHRH). This provides the potential to differentiate patients with pituitary dysfunction from patients with hypothalamus abnormalities. However, the routine use of the GHRH test is still being debated. The aim of this study was to assess the diagnostic usefulness of the GHRH test in the diagnostics of GHD.

Material and methods: The study group consisted of 20 prepubertal children with GHD. In all the children, one of the performed stimulation tests was the GHRH test.

Results: The results showed that the mean peak concentration of GH in the GHRH test was 14.7 ± 11.3 ng/mL. In eight children the MRI showed pituitary hypoplasia, in one patient pituitary hypoplasia and pituitary stalk agenesis, and in one patient septo-optic dysplasia. All patients with pituitary malformations, except for one patient with a hypoplastic pituitary gland, presented GH levels < 10 ng/mL in the GHRH test. The sensitivity of the GHRH test in the diagnostics of GHD was 45%.

Conclusions: The high correlation between the GHRH test and anatomical changes in the pituitary provides this test with a high predictive value. In individual clinical cases, knowledge about the level of damage in the hypothalamic-pituitary area can determine diagnostic and therapeutic procedures. (*Endokrynol Pol* 2015; 66 (2): 137-141)

Key words: growth hormone deficiency; GHRH test; short stature; children

Streszczenie

Wstęp: Testy stymulacyjne są podstawą procesu diagnostycznego niedoboru hormonu wzrostu (GHD). Jednym z takich testów jest test z somatoliberyną (GHRH). Umożliwia on odróżnienie pacjentów z zaburzeniami przysadki od pacjentów z zaburzeniami podwzgórza. Zasadność stosowania testu z GHRH w rutynowej diagnostyce GHD jest nadal dyskutowana.

Celem pracy była ocena przydatności diagnostycznej testu GHRH w diagnostyce SNP.

Materiał i metody: Grupę badaną stanowiło 20 dzieci prepubertalnych z GHD. U wszystkich dzieci jednym z przeprowadzonych badań był test GHRH.

Wyniki: Uzyskano następujące wyniki: średnie maksymalne stężenie hormonu w teście GHRH wynosiło $14,7 \text{ ng/ml} \pm 11,3$, u 8 dzieci stwierdzono hipoplazję przysadki, u 1 pacjenta hipoplazję przysadki i agenezję szypuły przysadki, u 1 — dysplazję przegrodowo-wzrokową. U wszystkich pacjentów z nieprawidłowością przysadki, z wyjątkiem jednego pacjenta z hipoplazją przysadki, stężenie GH w teście GHRH było < 10 ng/ml. Czulość testu GHRH w diagnostyce GHD wynosiła 45%.

Wnioski: Wysoka korelacja testu GHRH ze zmianami anatomicznymi w przysadce stanowi o wysokiej wartości predykcyjnej testu. W indywidualnych przypadkach klinicznych wiedza na temat poziomu uszkodzenia okolicy podwzgórzowo-przysadkowej może określić postępowanie diagnostyczne i terapeutyczne. (*Endokrynol Pol* 2015; 66 (2): 137-141)

Słowa kluczowe: niedobór hormonu wzrostu; test GHRH; niskorosłość; dzieci

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Introduction

The diagnostics of growth hormone secretion is based on stimulation tests using pharmacological and physiological provocation, assessing spontaneous GH secretion as 12–24 hours intermittent, or continuous blood sampling for GH and determination of the biochemical markers of GH action, such as IGF-1 (insulin growth

factor type 1) and IGFBP-3 (insulin growth factor binding protein type 3).

None of these tests is a perfect test, a so-called gold standard. Each test is associated with the risk of providing a false result. Therefore, in practice, diagnosis is not based on only a single test. The clinical picture and auxological data of a child are of great importance in the diagnostic process. The stimulation tests that have



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been used for over 30 years are performed to assess the pituitary GH reserve. The pharmacological stimulation tests use factors that stimulate the secretion of GH by the pituitary gland. Most of the stimulating factors that are used in these tests act via stimulating the pituitary gland by influencing the secretion of hypothalamic neurohormones, such as growth hormone releasing hormone (GHRH) and somatotropin release-inhibitory factor (SRIF). This mechanism is used in tests with insulin, clonidine, arginine, glucagon, and L-dopa [1]. This means that in these tests, assessment of GH secretion by the pituitary depends on the efficiency of the hypothalamus. A defect in the hypothalamus and/or the pituitary gland causes an abnormal response to those factors without distinguishing the level of damage. From the clinician's viewpoint, wishing to confirm GH deficiency, detecting the level of dysfunction is not critical.

The test utilising biosynthetic GHRH makes it possible to differentiate patients with pituitary dysfunction from patients with hypothalamus abnormalities. In individual cases, establishing the localisation of the damage may have an impact on diagnostic and therapeutic procedures.

The aim of the study was to assess the diagnostic usefulness of the GHRH test in the diagnostics of GHD in children.

Material and methods

The study comprised 20 children (12 girls, 8 boys) with diagnosed GHD in the Department of Paediatric Endocrinology and Rheumatology of Poznan University of Medical Sciences. The children were prepubertal, aged 4–16 years, htSDS was -3.06 ± 0.78 , and the BMI of the patients compared to age range was normal.

In all the children, one of the stimulation tests performed was the GHRH test. The sleep test (the estimation of spontaneous GH secretion during the first two hours of sleep, every 30 minutes) and at least two different pharmacological tests (with GHRH, insulin, clonidine, glucagon) were performed in each child. The GHD deficiency observed at peak GH concentration was < 10 ng/mL in the sleep test and in the two pharmacological tests. A peak GH concentration > 10 ng/mL in the GHRH test did not exclude GHD.

The protocol for the GHRH test was as follows; morning, fasting, GHRH (*i.v.* bolus) 1 mcg/kg. The blood samples were taken just before GHRH administration and at 15, 30, 45, 60, 90, and 120 minutes after GHRH administration.

The studied patients were not taking any medications, steroids, or other stimulants that might modify the GH secretion. All children admitted to the study were clinically and hormonally euthyroid.

Each patient with a diagnosed GH deficiency had magnetic resonance imaging (MRI) of the head performed. None of the patients presented a tumour. The anterior pituitary was considered hypoplastic when the height of the pituitary was less than two standard deviations compared to the normal ranges from Argyropoulou et al. [2]. The data obtained was analysed statistically.

Results

Mean peak concentration of GH in the GHRH test was 14.7 ± 11.3 ng/mL. The highest concentration was achieved at 30 minutes and was 11.33 ± 9.5 ng/mL.

Mean peak concentration of GH in the sleep test was 3.8 ± 1.86 ng/mL, in other pharmacological tests (no GHRH) — 5.6 ± 2.3 ng/mL.

In eight children the MRI showed pituitary hypoplasia, in one patient it showed pituitary hypoplasia and pituitary stalk agenesis, and in one patient septo-optic dysplasia. All the patients with pituitary malformation, except for the one patient with hypoplastic pituitary, showed GH levels < 10 ng/mL in the GHRH test. The sensitivity of the GHRH test in diagnosing GHD was 45%.

In nine children the GHRH test correlated with other stimulation tests. In 11 children with recognised GHD following sleep and pharmacological tests, the GHRH test showed GH secretion above 10 ng/mL.

The correlation between peak GH in the GHRH test and sleep test in the entire studied group described using the Pearson correlation coefficient was $r = 0.51$ and was statistically significant ($p < 0.05$). The correlation between peak GH in the GHRH test and other pharmacological stimulation tests was $r = 0.26$ and was not statistically significant ($p = 0.13$).

The results are presented in Table I and Figure 1.

Discussion

The results confirm the fact that the GHRH test should not be used as a basic test in GHD diagnostics in children. However, it is useful in organic hypopituitarism because there is a high correlation between the GHRH test and anatomical changes in the pituitary gland.

Abnormal synthesis, secretion, and the action of GHRH lead to growth hormone deficiency. Both congenital and acquired pathologies within the hypothalamus may be the cause of GHRH deficiency, followed by GH deficiency.

Acquired changes that may cause damage to the hypothalamus are: 1) cranio-cerebral trauma in childhood and perinatal trauma associated with hypoxia, forceps, and breech birth; 2) intracranial tumours such as cranio-

Table I. The characteristics of the studied group, GH concentration in tests performed, and the MRI picture
Tabela I. Charakterystyka badanej grupy, stężenia GH w wykonanych testach stymulacyjnych i obraz MRI

Patient	Age (years)	Ht SDS	Max GH concentration [ng/mL] in GHRH test	Max GH concentration [ng/mL] in sleep test	Max GH concentration [ng/mL] in other pharmacologic stimulation tests	Pituitary picture in MRI
1	14.0	-2.8	9.8	5.5	2.4 (I)	HP
2	5.0	-2.0	15.4	3.8	8.3 (C)	HP
3	13.0	-2.4	1.9	3.3	2.6 (G)	HP
4	5.5	-2.1	5.8	3.4	4.8 (G)	HP
5	8.0	-4.1	4.2	1.5	3.0(G)	HP + pituitary stalk agenesis
6	7.0	-2.8	28.0	3.7	6.7 (G)	N
7	6.5	-3.5	29.2	3.1	8.3 (C)	N
8	13.5	-3.0	43.3	7.0	5.2 (G)	N
9	6.7	-3.1	6.1	1.8	8.8 (G)	HP
10	16.0	-4.0	10.3	4.8	3.6 (G)	N
11	12.5	-2.7	1.9	2.5	4.2 (C)	HP
12	12.0	-3.0	35.3	4.7	5.8 (C)	N
13	4.0	-2.5	18.2	7.2	8.9 (G)	N
14	12.0	-2.0	6.3	1.6	6.0 (G)	SOD
15	4.5	-3.0	17.9	5.0	7.2 (C)	N
16	7.5	-2.7	17.7	6.9	4.9 (G)	N
17	16.0	-5.0	7.3	1.8	3.5 (I)	HP
18	12.0	-3.6	18.0	3.1	3.3 (C)	N
19	4.0	-3.0	12.0	1.1	5.3 (G)	N
20	6.0	-4.0	8.9	4.2	9.9	HP

C — clonidine; I — insulin; G — glucagon; HP — hypoplasia of pituitary anterior lobe, SOD — septo-optic dysplasia; N — normal picture

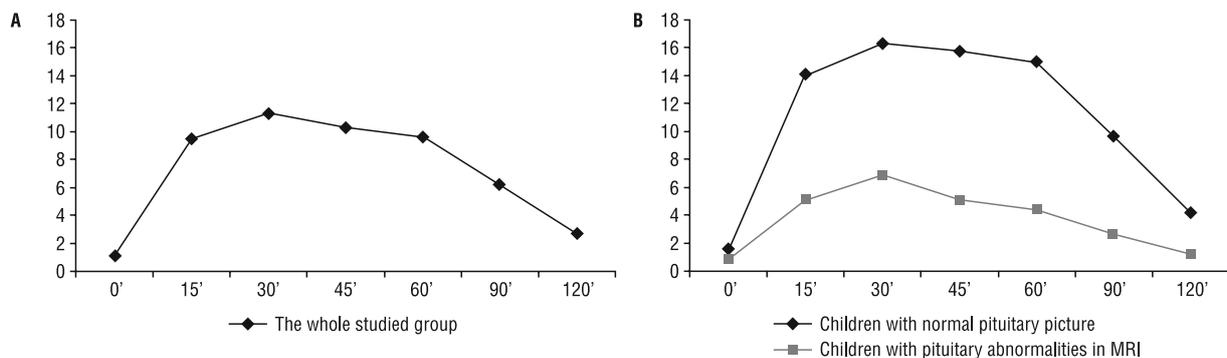


Figure 1. The GH [ng/mL] concentration at different times of blood sampling in GHRH test in the studied group (A). The GH [ng/mL] concentration at different times of blood sampling in GHRH test in children with pituitary abnormalities in MRI and in children with normal pituitary picture (B)

Rycina 1. Stężenie GH [ng/mL] w różnych oznaczeniach czasowych w teście GHRH w badanej grupie dzieci (A). Stężenie GH [ng/mL] w różnych oznaczeniach czasowych w teście GHRH u dzieci z nieprawidłowościami w przysadce w MRI i u dzieci z prawidłowym obrazem przysadki (B)

pharyngioma, germinoma, optic glioma, histiocytosis X, and the state after operation of a brain tumour; 3) inflammation within the cranial cavity (bacterial, viral, fungal, parasitic aetiology); 4) cellular infiltration within the CNS (sarcoidosis, tuberculosis, haemochromatosis);

5) cardiopulmonary vascular disorders; and 6) status after post-radiotherapy or chemotherapy.

The type of congenital GHRH deficiency is mainly determined by organic lesions. Organic lesions occur due to anencephaly and midline defects of the brain,

such as holoprosencephaly, septo-optic dysplasia, cleft lip and palate, abnormal dentition of the upper jaw (single central incisor), and herniation of the base of the brain. Abnormal development of the midline organs can lead to different types of birth defects within the hypothalamus and pituitary glands [3]. Abnormal function of the hypothalamus and pituitary may be caused by a classic triad of congenital malformations — ectopic posterior pituitary lobe, hypoplasia or aplasia of anterior pituitary lobe, and pituitary stalk interruption or agenesis. This is a congenital syndrome that is relatively frequently observed in MRIs of the head in growth hormone-deficient patients [4]. There are also patients described with short stature due to a GHRH-R defect, e.g. homozygous mutations in the GHRH receptor gene [5]. Mutations in the gene encoding the GHRH molecule have not yet been detected, which may suggest that such defects generate not only GH deficiency, but perhaps do not allow proper development of the pituitary.

In our examined group, the observed malformations of the pituitary were congenital. The most frequent was hypoplasia of the pituitary anterior lobe.

One patient had hypoplasia of the anterior lobe, associated with agenesis of the pituitary stalk. The next patient had a rare syndrome — septo-optic dysplasia. All patients with pituitary malformations, except for the one with a hypoplastic pituitary gland, presented GH below the range in the GHRH test. This shows the high sensitivity of the GHRH test in detecting pituitary malformations. Changes in pituitary shape and size may correlate with gland activity, particularly growth hormone secretion. There is evidence directly linking pituitary height and stature between the age of 8 days and 21 years, and a correlation between reduced or absent anterior lobe with GH deficiency [2,6]. This study proves that the height of the pituitary gland modifies secretion of GH. Patients with pituitary hypoplasia and GH secretion close to or above 10 ng/mL may suggest that the volume of the pituitary gland is not the only determinant of pituitary function. The accuracy of the MRI examination can also sometimes be faulty. Correct interpretation of MRI scans requires an understanding of the specific features of the paediatric pituitary gland, how it differs from the adult gland and changes during development.

Since there is a biosynthetic analogue of GHRH, the neurohormone is used in diagnostics. On the pharmaceutical market there are now three analogues of GHRH: GHRH (1–40), GHRH (1–44), and GHRH (1–29) NH₂. The last of these is recommended for diagnosis and treatment [1].

Studies in healthy children and adults have shown that GH concentrations after intravenous bolus injection of GHRH analogue increases after five minutes

and reaches maximum concentration between 30 and 60 minutes [1]. The amounts of GH secreted from the pituitary are GHRH dose dependent [7]. The best response to GHRH is likely to be reached at a dose of 1 mcg/kg [8], so this was the dosage used in our group. The highest level of GH was observed 30 minutes after GHRH administration.

The studies showed no significant effect of the stage of sexual maturation on concentrations of GH in the GHRH test [9, 10]. The use of oestrogen priming did not change the response of GH after GHRH [11]. This fact suggests that sex steroids influence growth hormone secretion by modifying the secretion of hypothalamus neurohormones. This is a definite advantage of GHRH compared to other stimulants used in tests. It has been proven that GH peaks after agents acting via the hypothalamus are lower in the prepubertal period compared to puberty. It can cause false positive results. We tried to choose a very homogenous group, to eliminate eventual false results. This is why our study group was prepubertal and the BMI was normal, comparing age and sex ranges. The prepubertal status of even older children was probably caused by delayed bone age and low concentration of gonadotropins.

Side effects during the test are reported very rarely. The most common symptoms after administration of GHRH are: temporary feeling of warmth, hot flushes, a metallic taste in the mouth, slight nausea, vomiting, headache, and chest pain. These symptoms are transient and disappear quickly. Our children did not report any adverse events.

A GH threshold equal to 10 ng/mL, widely used in stimulation tests, is also applicable to the GHRH test. There are many studies on the optimal cut-off point for GH in GHRH test [11]. Ranke estimated GH concentration after GHRH administration in 86 healthy children. The normal range specified in the ± 2 SD for maximum GH was from 11.8 to 172.4 ng/mL. Based on these results, Ranke found a GH threshold equal to 10 ng/mL to be the most diagnostically useful [12]. This cutoff value was also used in our study in all performed tests. Other studies have shown that for a GH cut-off point of 7–10 ng/mL after GHRH, the specificity of the test is higher than 95%, with a sensitivity of 30% [12]. In children with growth hormone deficiency caused by organic reasons, GH peaks are lower than in patients with idiopathic hypopituitarism [12]. Low GH after stimulation with GHRH indicates GHD with high probability, and normal GH levels do not exclude GHD. Therefore, this test is not often used as a routine diagnostic test. The GHRH test is used more for diagnosing the cause of GHD and to identify the lesion site (pituitary vs. hypothalamus).

Due to the fact that GHD in children is largely caused by hypothalamic reasons, the GHRH test, ac-

ording to some authors, has limited usefulness. It was found that 76% of children with partial GHD and 39% with total GHD identified on the basis of routine tests, in the GHRH test presented GH peaks above 10 ng/mL [1].

Patients with normal GH secretion in the GHRH test but with a low GH in tests such as with clonidine or L-dopa are evidence that the background of abnormal growth is caused by GHRH deficiency, and therefore they should be treated with recombinant GH. The presented study confirms that normal GH level in GHRH test does not exclude hypopituitarism — 11 children (55%) out of 20 had GH > 10 ng/mL, all with recognised GHD.

The sensitivity of the GHRH test in our group was 45%. GH levels above 10 ng/mL in the GHRH test did not exclude GHD. The test correlated in 9 patients out of 20 with other tests. Pearson's *r* for correlation between GHRH test with sleep test was relatively high ($r = 0.51$) and statically significant. It suggests that the physiological stimulation test reflects the function of the pituitary gland and correlates with pituitary malformations. Pearson's *r* for correlation between GHRH test with other pharmacologic tests was relatively low ($r = 0.26$, $p = 0.13$). It confirms that the usefulness of GHRH test in GHD diagnostics is restricted but has very high prediction level in patients with organic reasons for GHD.

In patients after radiotherapy there is no correlation between the test with GHRH and conventional tests. In 80% of patients the GH secretion was higher after stimulation with GHRH than after arginine and insulin [1]. This shows that radiation damage takes place in the first place in the hypothalamic regulatory mechanism. With time, after radiation, the response to GHRH becomes weaker. This may be due to the damaging effects of radiation on somatotrophs or the hypofunction of these cells because of a chronic lack of stimulation by GHRH. In the case of prolonged inactivity of the hypothalamus and lack of pituitary gland stimulation, the GHRH test can give false positive results. Some authors advocate the use of priming with GHRH for a week prior to the test with GHRH, if damage to the hypothalamus is suspected [13].

Conclusions

Due to its low sensitivity and low correlation with other tests, the GHRH test has a low diagnostic usefulness in identifying growth hormone deficiency. However,

due to its very high specificity and small amount of side effects, it may be useful as one of the tests applied, especially when an organic reason for hypopituitarism is suspected. The high correlation between the GHRH test and anatomical changes within the pituitary gland gives the test a high predictive value. In individual clinical cases, knowledge about the site of damage in the hypothalamic-pituitary area can determine the diagnostic and therapeutic procedures. On the other hand it should be emphasised that a normal GH concentration in the GHRH test does not exclude pituitary insufficiency, due to the frequently occurring hypothalamic aetiology of GH secretion disorders in children.

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