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GHRH plus arginine and arginine administration evokes the same ratio of GH isoforms levels in young patients with Prader-Willi syndrome

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ABSTRACT

Human GH is present in pituitary and circulation as several isoforms, the prevalent being 22 kDa- and 20 kDa-GH. Recently, we have demonstrated the preservation of a normal balance in GH isoforms after GH releasing hormone (GHRH) plus arginine (ARG) administration in adult patients with Prader-Willi syndrome (PWS), one of the most common causes of syndromic obesity, often associated with GH deficiency (GHD). Aim of the present study was to measure circulating levels of 22 kDa- and 20 kDa-GH in young PWS patients (n = 24; F/M: 10/14; genotype UPD/DEL/met +: 11/11/2; age: 10.8 ± 5.3 years; BMI SDS: 2.0 ± 2.0; GHD: 16/24; obesity: 12/24) after combined GHRH + ARG or ARG administration. The results were analysed subdividing the GHRH + ARG and ARG groups on the basis of PWS genotype, GHD status and obesity. Circulating levels of 22 kDa- and 20 kDa-GH were measured by a chemiluminescent or fluorescent method based on specific pairs of monoclonal antibodies.

GHRH + ARG or ARG significantly stimulated the secretion of 22 kDa-GH but not that of 20 kDa-GH in all PWS patients. No significant GHRH + ARG- vs. ARG-induced changes in the ratios of 22 kDa- to 20 kDa-GH peaks were observed in all PWS patients, although 22 kDa- or 20 kDa-GH peaks were significantly higher in the GHRH + ARG than ARG group. When subdividing PWS patients in UPD vs. DEL, obese vs. non obese and GHD vs. non GHD subgroups, GH peaks were significantly higher in nonobese than obese patients and in non GHD than GHD patients administered with either GHRH + ARG or ARG test, apart from the comparisons in the DEL/UPD subgroups. Anyway, the ratios of peak levels of 22 kDa- to 20 kDa-GH were similar after GHRH + ARG vs. ARG in all subgroups investigated.

In conclusion, this study shows that administration of two different pharmacological tests, i.e. ARG, capable of reducing hypothalamic somatostatinergic tone, and GHRH (+ ARG), that directly acts at pituitary level on the somatotropic cell, evokes the same ratios of GH isoforms in young PWS patients, suggesting that the hypothalamic dysfunction in this genetic disorder does not alter the qualitative and quantitative composition of GH isoforms present in circulation.

1. Introduction

Prader-Willi syndrome (PWS) is a complex multisystemic genetic disorder caused by lack of expression of genes on the paternally inherited chromosome 15q11.2-q13 region. Three main genetic subtypes have been characterized in PWS: paternal 15q11-q13 deletion (DEL: 65–75% of cases), maternal uniparental disomy 15 (UPD: 20–30% of cases), and imprinting defects (met +: 1–3%) [1].

Clinical manifestations change with patient's age, hypotonia, poor sucking and failure to thrive being evident during infancy, while short stature, hyperphagia with food seeking and excessive weight gain, several endocrinopathies, developmental delay, cognitive disabilities

Abbreviations: PWS, Prader-Willi syndrome; GHRH, GH releasing hormone; ARG, arginine; kDa, kiloDalton; BMI, body mass index; sds, standard deviation score; mAb, monoclonal antibody; CV, coefficient of variation; IGFBP-3, IGF binding protein 3

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and behavioral being problems in the childhood/adolescence [2].

The predominant aetiopathogenetic hypothesis is that PWS phenotype is the result of a "hypothalamic dysfunction", which is responsible for hyperphagia, temperature instability, high pain threshold, hypersomnia and multiple endocrine abnormalities including GH and thyroid-stimulating hormone deficiencies, hypogonadism and central adrenal insufficiency [3].

As far as GH axis is concerned, spontaneous and stimulated GH secretion is reduced, a condition often associated with low IGF-I levels [4,5]. Reportedly, GH deficiency (GHD) is found in 80% of children [6], while 8–55% of adults meet criteria for severe GHD [7–9]. These percentages widely vary because of the different provocative tests and/or cut-off limits adopted in the single clinical studies. In addition, because of the high prevalence of obesity in PWS, permanent GHD due to the hypothalamic(-pituitary) dysfunction in PWS may be difficult to distinguish from the reversible blunting of GH release in obese patients [10]. Finally, PWS genetic subtypes seem to influence GH response to the stimulation tests, with a higher incidence of GHD in UPD than DEL patients [11,12].

Human GH is present in pituitary and circulation as several isoforms. This biochemical heterogeneity is due to the existence of different genes (GH1 or GH-N and GH2 or GH-V), mRNA splicing, post-translational modifications and peripheral metabolism after hormonal secretion. In particular, alternative mRNA splicing of the GH1 transcript yields two products: 22 kiloDalton (kDa)-GH (the main pituitary and circulating GH form) and 20 kDa-GH. Anyway, many other minor post-translationally modified GH forms, either monomers or oligomers, exist. Some of these isoforms, such as 22 kDa- and 20 kDa-GH, can be measured by analytical methods based on the use of monoclonal antibodies that specifically recognize unique or common portions (epitopes) of GH molecules [13].

Recently, we have demonstrated an unaltered ratio of 22 kDa- to 20 kDa-GH in PWS adults when administered with GH releasing hormone (GHRH) + arginine (ARG) [14]. However, a limitation of our previous study was the use of a potentiated GH releasing stimulus, which did not allow ruling out that the supposed hypothalamic dysfunction in PWS might cause an unbalance of these circulating isoforms. This occurrence can be disclosed by using a stimulus acting only at the hypothalamic level. Thus, the aim of the present study was to measure circulating levels of 22 kDa- and 20 kDa-GH in young PWS patients after ARG, a "pure" hypothalamic stimulus, capable of reducing somatostatinergic tone, or GHRH + ARG, a potent pharmacological test evaluating the total GH pituitary reserve [15–17]. Based on the previous considerations, the results were analysed taking into account the three main factors affecting GH responsiveness in PWS, i.e. genotype, obesity and GHD.

2. Materials and methods

Being the present study a continuation of a previous research (see the Introduction), the methods (i.e., administration of endocrinological tests or biochemical measurement of GH isoforms) are similar to those reported in one of our recently published works [14].

2.1. Patients

Twenty-four PWS young patients, 14 males and 10 females, aged 2.1–18.8 years (mean ± SD: 10.8 ± 5.3 years), recruited at the Autoimmune Endocrine Diseases Unit, Bambino Gesù Children's Hospital, Research Institute, Rome, Italy, were included in the study. All patients showed the typical PWS clinical phenotype. Cytogenetic analysis was performed in all subjects: 11 had UPD and 11 DEL, while met + was found in the remaining 2 individuals. Standing height was determined by a Harpenden Stadiometer (Holtain Ltd., Dyfed, UK), and expressed as standard deviation score (SDS) for height (HSDS), according to the published WHO growth standards [18,19]. In our population, HSDS ranged from −1.8 to 1.3 (mean ± SD: −1.3 ± 1.2). Body weight was measured to the nearest 0.1 kg, by using standard equipment. BMI was defined as weight in kilograms divided by the square of height in metres. Mean (± SD) BMI was 26.1 ± 9.8 kg/m^2 (range: 18.4–32.6 kg/m^2), while mean (± SD) BMI SDS was 2.0 ± 2.0 (range: −0.5–6.3). At the time of the study, no subjects were undergoing sex steroid substitution. Seventeen PWS subjects had previously undergone GH treatment, withdrawn in all cases at least 2 years before starting the study protocol.

Patients were diagnosed as having GHD or non GHD, according to a peak stimulated GH of < 10 μg/l after ARG or < 20 μg/l after GHRH + ARG [20], as described in details below. Patients were considered obese when his/her BMI SDS was > 2.0 [21].

The entire study protocol was approved by the ad hoc Ethical Committee of Bambino Gesù Children's Hospital and that of Istituto Auxologico Italiano (trial registered with ref. no. 01C627 and acronym ISOGHBAPWS). Written informed consent was obtained from all participants by their parents.

2.2. Endocrine protocol

Twelve subjects underwent a GHRH + ARG test, while the response to ARG alone was determined in the remaining 12 patients. All tests started at 8:30 AM after overnight fasting, with the patients recumbent. Fifteen minutes after an indwelling catheter had been placed in an antecubital vein, each subject received GHRH (1–29) injection (GHRH, Ferring GmbH, Kiel, Germany; 1 μg/kg as i.v. bolus at 0 min) (GHRH + ARG group) or no injection (ARG group). From 0 to 30 min, 0.5 g/kg (maximum dose 30 g) of ARG hydrochloride (SALF, Bergamo, Italy) was infused in all patients. Blood samples for GH determination were drawn at 0, 30, 45, 60, 90 and 120 min. In addition, serum levels of IGF-I and IGFBP-3 were determined in the sample drawn at 0 min. IGF-I was expressed both as absolute values and SDS.

2.3. Assays

For diagnosing GHD, serum GH levels were determined by a commercially available immunometric chemiluminescence kit (Immulite 2000, DPC, Los Angeles, CA, USA). The international standard for recombinant human GH NIBSC Code 98/574 was used as standard material. Intra- and inter-assay coefficients of variation (CVs) for this assay were 2.5% and 6%, respectively. The sensitivity of the method was 0.01 μg/l.

22 kDa-GH was measured using the automated IDS iSYS hGH chemiluminescence assay system. In this assay, the detection monoclonal antibody (mAb) targets an epitope in the loop connecting helix 1 and 2 of GH, which is missing in 20 kDa-GH, thereby conferring specificity of the assay for the 22kD-GH molecules. Details have been published in a previous work [22]. In our hands, functional sensitivity of the assay is 0.04 μg/l and intra- and inter-assay CVs both are below 5%.

The 20 kDa-GH was measured using an in-house time resolved fluorescence assay as described before [23]. The assay employs two mAbs with no cross-reactivity to 22kDa-GH; intra- and inter-assay CVs were 5.4% and 6.3% at 0.2 μg/l and the functional sensitivity was 0.025 μg/l.

Serum concentrations of IGF-I were determined by chemiluminescence (Liaison IGF-I immunoassay, DiaSorin, Saluggia, VC, Italy), with intra- and inter-assay CVs of 4·8% and 6·7%, respectively.

Serum levels of IGFBP-3 were measured by immunochemiluminescence (Liason Nichols Advantage, San Juan Capistrano, CA, USA), with inter-assay and intra-assay CVs of 4.8% and 5%, respectively.

2.4. Statistical analysis

The Sigma Stat 3.5 statistical software package was used for data