NON-SUPPRESSIBLE INSULIN-LIKE ACTIVITY IN LARON'S SYNDROME

By

Knud W. Kastrup and Jürgen Zapf

ABSTRACT

Severe growth retardation is found in patients with high levels of growth hormone and low sulphation factor activity or somatomedin. Also non-suppressible insulin-like activity (NSILA-s) has been found to be very low in a patient with this condition as measured by bioassay, protein binding assay and radioimmunoassay and to be below activities found in hypopituitary patients. Partially purified NSILA-s restored the ability of serum to increase sulphation activity although full restitution may still depend on other factors. These findings support the hypothesis that NSILA-s belongs to the family of somatomedin and thus is involved in promoting growth, and that low activity of these growth factors is a primary cause of the growth retardation found in these patients.

Growth retardation in patients with elevated levels of immunoreactive growth hormone has first been described by Laron et al. (1966). The syndrome seems to be caused by a generalized defect in GH receptors which leads to decreased generation of somatomedins (SMs) (Jacobs et al. 1976), a family of substances that is thought to mediate at least some of the actions of growth hormone on its target tissues (Daughaday et al. 1972). Indeed, low levels of bioassayable SM have been found in these patients (Daughaday et al. 1969; Laron et al. 1971; Kastrup et al. 1975). In contrast to patients with GH deficiency, GH treatment of Laron dwarfs does not stimulate skeletal growth and it does not cause an increase in plasma activity.

NSILA-s designates small molecular weight non-suppressible insulin-like activity extracted from whole serum by a one-step chromatographic procedure.
on Sephadex G-50 at acidic pH (Schlumpf et al. 1976; Zapf et al. 1977). In previous work NSILA-s had been extracted with acid/ethanol from precipitate B obtained from human plasma by a modified Cohn fractionation (Bürgi et al. 1966) and further purified to a specific biological activity of 5–200 mU/mg of protein (standardized in the rat fat pad assay with insulin as the reference). The final purification yields two chemically (Rinderknecht & Humbel 1976a,b, 1978a,b) and biologically (Zapf et al. 1978c) closely related polypeptides of molecular weight 7500, designated as insulin-like growth factors (IGF) I and II.

Non-suppressible insulin-like activity (NSILA-s) of human serum is a well characterized member of the SM-family (Zapf et al. 1978b; Chochinov & Daughaday 1976).

As for SMs, elevated levels of NSILA-s have been found in acromegalics and decreased levels in hypopituitary patients (Schlumpf et al. 1976; Zapf et al. 1977). However, no studies of NSILA-s have been reported in Laron’s syndrome. We have, therefore, determined NSILA-s by various assay techniques, in one of these patients and studied the effect of the patient’s serum on sulphate incorporation into chick cartilage both in the presence and absence of partially purified NSILA-s.

MATERIALS AND METHODS

The patient studied was a 10 year old boy with severe growth retardation and clinical features of Laron’s syndrome. Further observations and metabolic studies in this patient have been published previously (Kastrup et al. 1975). The clinical features are indistinguishable from those found in patients with lack of growth hormone. The bone age was severely retarded. The height was 96 cm corresponding to the mean height for 3 years old normal boys. Fasting growth hormone levels were elevated and in the range of 80 ng/ml. From early infancy he had suffered from hypoglycaemic spells, but insulinopenic responses were found after glucose tolerance and arginine test, whereas cortisol response to hypoglycaemia was normal (peak value: 38.7 μg/100 ml).

Hands, feet and face were small with a prominent forehead. He was slightly obese with small genitalia and had a peculiar high-pitched voice. The parents were first cousins. Although small genitalia are commonly found in these patients they later undergo a normal pubertal development (Laron et al. 1972). From the observations mentioned above, it can be assumed that the production of ACTH and gonadotrophins is within normal limits, whereas the disturbance in the metabolism of carbohydrate metabolism is not yet fully understood.

Determination of NSILA-s. – NSILA-s was extracted from whole serum by Sephadex G-50 chromatography in 1 m acetic acid (Schlumpf et al. 1976; Zapf et al. 1977). This procedure dissociates and separates small molecular weight NSILA-s from its specific carrier protein (Zapf et al. 1977). Fractions eluting between 55 and 80 % bed volume were pooled and lyophilized as described earlier (Zapf et al. 1977) and NSILA-s was determined.
a) in the fat pad assay (Froesch et al. 1963)

b) by a protein binding assay, as described in detail elsewhere (Zapf et al. 1977)

c) by a radioimmunoassay using antiserum against NSILA-s, identical to that used by Reber & Liske (1976) and kindly supplied by Dr. Reber, Hoffmann La Roche, Basel. Its cross-reactivity with IGF II is 1/30 of that with IGF I. To 0.2 ml of a 1:10000 dilution of the antiserum in 0.1 m phosphate buffer / 0.2 % HSA, pH 7.0, was added 0.1 ml of [¹²⁵I]IGF I (20 000 cpm, diluted with the same buffer) and 0.1 ml of IGF I standard (0.2–50 ng) or appropriately diluted samples of the 55–80 % lyophilized pool (see above). After a 12 h incubation at 4°C, 0.1 ml of a charcoal suspension (2% charcoal equilibrated in 0.1 m phosphate buffer / 2 % HSA, pH 7.0, for at least 6 h) was added and incubation continued for 20 min at 0°C. The charcoal was then centrifuged off (10 min, 2500 g) and 0.5 ml of the clear supernatant was counted in a gamma-counter (Packard).

IGF I and partially purified NSILA-s (4.5 mU of insulin equivalents/mg, containing an approximate 1:1 mixture of IGF I and II) were kindly supplied by Drs. Rinderknecht and Humbel. The iodination procedure for IGF I has been described recently by Zapf et al. (1978a). Partially purified NSILA-s was standardized in the rat fat pad assay (Froesch et al. 1963) with insulin as the reference. The intra-assay (n = 15) and inter-assay coefficients of variation were 6 ± 1 and 13.3 ± 2.2 %, respectively.

**Determination of sulphation activity.** The chick embryo pelvic cartilage assay was performed with diluted whole serum according to Hall (1970) with slight modifications. Incubation time was prolonged to 18 h in Eagle's medium with 35S during the whole incubation. Samples were assayed in quadruplicate in a 4 point assay or in single determinations with various serum concentrations as given in Figs. 1 and 2. The reference serum was a pool of serum from normal children aged 10–12 years. To avoid inter-assay variation all samples were analyzed in the same assay. The intra-assay coefficient of variation was found to be 11 %, and the index of precision (lambda) was for 20 consecutive determinations 0.15 ± 0.07.

**R E S U L T S**

The results of the NSILA-s determinations and the sulphation activity of the patient's serum are given in Table 1 together with the normal ranges and the ranges found in hypopituitary patients.

NSILA-s as determined in the fat pad and in the protein binding assay was markedly decreased (< 25 and 23 μU/ml, respectively) in the patient with Laron's syndrome. The values were lower than in hypopituitary patients. This was even more pronounced when IGF I was measured in the radioimmunoassay (1.5 ng/ml). Sulphation activity in the patient's serum was 1/3–1/4 of normal activity of the age.

In Fig. 1 dose-response curves of sulphation activity obtained with different concentrations of normal and of the patient's serum are compared with the dose-response curves obtained by increasing the NSILA-s-concentrations from 7.5–30 μU/ml in either 10 or 20 % patient's serum. Addition of partially puri-
Table 1.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Laron's syndrome</th>
<th>Normal</th>
<th>Hypopituitary patients</th>
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<tr>
<td>NSILA</td>
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<tr>
<td>1) Fat pad assay</td>
<td>&lt; 20 μU/ml</td>
<td>143 ± 45 μU/ml</td>
<td>61 ± 13 μU/ml</td>
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<td>(n = 33)</td>
<td>(n = 33)</td>
<td>(n = 10)</td>
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<tr>
<td>2) Protein binding assay</td>
<td>23 μU/ml</td>
<td>320 ± 67 μU/ml</td>
<td>165 ± 57 μU/ml</td>
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<td>(n = 47)</td>
<td>(n = 47)</td>
<td>(n = 16)</td>
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<tr>
<td>3) Radioimmunoassay (IFG I)</td>
<td>1.5 ng/ml</td>
<td>148 ± 45 ng/ml</td>
<td>22 ± 7 ng/ml</td>
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<td>(n = 13)</td>
<td>(n = 13)</td>
<td>(n = 8)</td>
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Somatomedin

Chick embryo pelvic cartilage

0.28 U/ml 0.91 ± 0.15 U/ml 0.47 ± 0.08 U/ml
(n = 15) (n = 15) (n = 8)

Non-suppressible insulin-like activity and somatomedin in a patient with Laron's syndrome compared with hypopituitary patients and normal controls (means ± sd).

STIMULATION OF SULFATE INCORPORATION

![Graph showing dose-response curves](Fig. 1.)

Dose-response curves obtained with normal serum and with serum from a patient with Laron's dwarfism without and with the addition of NSILA-s in increasing concentrations.

Acta endocr. 90, 3
The effect of NSILA-s alone and after addition to serum from a patient with Laron's dwarfism compared to the sulphation activity of normal human serum.

NSILA-s increased the stimulation of sulphate incorporation in a dose-dependent manner. The NSILA-s dose-response curves in the presence of 10 or 20% patient's serum were identical and they were parallel to the dose-response curve carried out with increasing concentrations of normal serum.

NSILA-s alone (7.5 and 30 µU/ml) as well as 7.5 µU/ml of NSILA-s + 10 or 20% of the patient's serum had only a small stimulatory effect according to the chick cartilage assay (Fig. 2). Thirty µU/ml of NSILA-s in the presence of 10 or 20% patient's serum was as effective as 10% normal serum. Addition of higher amounts of NSILA-s to the patient's serum caused only a slightly higher stimulation from which it can be concluded that 30 µU/ml gave near maximal stimulation.

**DISCUSSION**

The low NSILA-s level found in the serum of the patient with Laron's syndrome corresponds to the decreased SM activity (Table 1). Compared to the NSILA-s levels in hypopituitary patients the NSILA-s level in the serum from
the patient was even more drastically reduced, as was IGF I, which was barely
detectable. Since 1 ng of pure IGF I or II corresponds to 0.3 μU of insulin-
equivalents as standardized in the fat pad assay (Zapf et al. 1978b), and since
the fat pad assay in serum extracts gives approximately 50 % lower NSILA-s
values than the protein binding assay (Zapf et al. 1977), IGF I accounts for
5 % of the total extractable NSILA-s of the patient’s serum compared to 11 %
in hypopituitary (Table 1), 28 % in normal (Table 1) and 70 % in acromegalic
sera (Zapf et al. 1978c). Thus marked decrease in small molecular weight
NSILA-s is involved in promoting growth. Furthermore, the comparison of
IGF I levels in normal, hypopituitary and acromegalic patients and in the
patients with Laron’s syndrome suggests that IGF I may be more important
in this respect than IGF II. This is also indicated by the finding that IGF I,
at low concentrations, is a more potent sulphation factor in the rat cartilage
assay than IGF II (Zapf et al. 1978d).

The observation that, in contrast to the rat cartilage assay, NSILA-s alone
is only a weak sulphation factor in the chick cartilage assay (Fig. 2) and
apparently requires the presence of another, GH-independent serum factor has
also been described by Froesch et al. (1976) and by Laron (1977). However, in
the presence of 10 or 20 % serum from the patient, which, by itself, contains
only very low sulphation activity (Table 1, Fig. 1), NSILA-s stimulates sul-
phation in a dose-dependent manner (Fig. 1) and thus restores the ability of
the serum to increase sulphation. Although full restitution of the sulphation
activity in the patient’s serum may still be dependent on other factors, the
presence of NSILA-s in serum seems to be an essential prerequisite for the
stimulation of sulphate incorporation into cartilage tissue.

It is interesting that total NSILA determined in whole serum by the fat
pad assay is not different in normal, hypopituitary and acromegalic subjects
and that differences become apparent only after acidic chromatography of the
sera (Schlumpf et al. 1976). This was also observed with the serum of the Laron
patient (not shown). These findings are consistent with the recent observation
that, besides small molecular weight NSILA-s bound to its carrier protein
(Zapf et al. 1975), serum contains considerable amounts of large molecular
weight NSILA non-dissociable by acid treatment, which is active in the fat
pad assay but not in the sulphation assay (Poffenbarger 1975; Zapf et al. 1978a)
and which does not appear to be a GH-dependent growth factor. Thus, acid
chromatography of serum is essential for the subsequent measurement of GH-
dependent small molecular weight NSILA-s.

Lastly, it should be pointed out that not only IGF I and II, but also somato-
tomedin A (SM-A) and somatomedin C (SM-C) are active in the fat pad and
in the protein binding assay, as well as in other biological and radioreceptor
assays presently available (Zapf et al. 1978d). However, recent results obtained
in the IGF I and a SM-C radioimmunoassay are compatible with the possi-
bility that SM-C may be identical with IGF I (Zapf, unpublished results; van Wyk, personal communications). The high degree of cross-reactivity between SM-A and IGF I in the IGF I and SM-A radioimmunoassay (Zapf, unpublished results; Hall et al. 1978) leave a similar possibility open.

REFERENCES


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420