NON-SUPPRESSIBLE INSULIN-LIKE ACTIVITY
DURING ACUTE METABOLIC
AND ENDOCRINE CHANGES IN DOGS

By

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ABSTRACT

Non-suppressible insulin-like activity (NSILA-S) was determined in dogs after acute changes in the blood sugar and after injection of growth hormone. Serum was chromatographed over Sephadex G-50 columns equilibrated in 1 m acetic acid and NSILA-S was determined in the fractions between 55 and 85% column volume using a protein binding assay and the conventional fat pad assay. The results obtained with these two methods correlated rather well (r = 0.74). Hyperglycaemia induced by an intravenous glucose load, by intravenous administration of mannheptulose or both was not followed by an increase in NSILA-S levels. The injection of insulin and human growth hormone did not lead to alterations of the NSILA-S levels. It is concluded that total NSILA-S levels in the dog do not change acutely following manipulations of the blood sugar and that, in all likelihood, NSILA-S plays no role in the regulation of blood glucose.

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The insulin-like effects of non-suppressible insulin-like activity (NSILA-S) have been extensively studied in vivo and in vitro (for review see Oelz et al. 1972). In more recent studies it has been demonstrated that NSILA-S exerts not only insulin-like effects in the classical sense but also marked growth promoting effects (Morell & Froesch 1973) and that NSILA-S is a potent sulphation factor (Zingg & Froesch 1973; Froesch et al. 1976). However, the physiological significance of NSILA-S remains to be elucidated. In 1975 (Zapf et al. 1975b) we demonstrated the presence in serum of a highly specific carrier protein for NSILA-S and Kaufmann et al. (1977) found that the half-life of NSILA-S in the rat is of the order of magnitude of 4 h. Since more than 95% of NSILA-S in the circulation is present in the bound form, which has this rather long half-life the finding of Megyesi et al. (1975) who described acute changes in total NSILA-S in the serum of human subjects came rather as a surprise.

The experiments, the results of which are described in this study were carried out in order to verify whether or not acute changes in the NSILA-S levels can be obtained by acute changes in blood sugar and by a single injection of growth hormone.

MATERIALS AND METHODS

Mannoheptulose (Sigma) was infused as a solution of 20%. Human growth hormone (Serono) containing 2 U/mg was dissolved in saline (pH 9) in a concentration of 0.2 mg/ml. Crystalline pork insulin (Actrapid®, Novo) was diluted with sterile saline to a final concentration of 1 U/ml before injection. Glucagon-free pork insulin (Hoechst) was used as a standard in the rat fat pad assay (Froesch et al. 1963). Highly purified NSILA-S was labelled with 125I and a preparation containing 4.5 mU/mg was used as standard in the protein binding assay (Zapf et al. 1977). Both were a generous gift from E. Rinderknecht and R. E. Humbel, Department of Biochemistry, University of Zurich. NSILA-S was extracted from individual sera by the method of Schlumpf et al. (1976), with the exception that Sephadex G-50 was found to give a better separation of NSILA-S from its binding protein than Sephadex G-75. Using this method, NSILA-S appears between 55 and 85% of total bed volume. The fractions in this region were pooled, lyophilized, rinsed with 10 ml of 0.1 M NH4HCO3, re-lyophilized and their NSILA-S content was then determined.

For the fat pad assay we used male ZBZ Cara rats weighing 120–130 g (Froesch et al. 1963). In addition, NSILA-S was also determined in the protein binding assay (Zapf et al. 1977). All experiments were carried out using 5 adult, pure bred female Labrador dogs weighing 23.4 ± 2.1 kg (mean ± standard error). The dogs were a generous gift from Dr. W. Rossbach, Hoffmann-LaRoche. All experiments on these dogs were carried out after an overnight fast. Injections and blood collections were performed by puncture of the antebrachial cephalic vein. Blood samples were allowed to clot for 2 h at 4°C, centrifuged at 4000 r.p.m. and the serum was stored at −20°C.

For statistical analysis Student’s t-test and linear regression were employed.
Correlation between NSILA-S values determined in the protein binding assay (ordinate) and in the rat fat pad assay (abscissa). Serum was chromatographed over Sephadex G-50 and NSILA-S was determined in a pool of the fractions eluted between 55% and 85% (see Materials and Methods) \( y = (20 \pm 24) + x (0.88 \pm 0.10), r = 0.74. P < 0.001, n = 66. \bullet = \text{hypophysectomized dogs, } \bigcirc = \text{normal dogs.}

**RESULTS**

Sephadex G-50 chromatography in 1 M acetic acid of serum yields NSILA-S quantitatively in the fractions eluted between 55 and 85% of total bed volume. Fig. 1 shows the rather good correlation between the NSILA-S values obtained by the pad assay and the protein binding assay in 66 sera. The correlation coefficient is 0.74. The concentration of NSILA-S showed considerable variation from dog to dog and from day to day: 228 ± 25, 234 ± 31, 243 ± 27, 261 ± 22 and 343 ± 15 μU/ml (mean ± SE, n = 5).

The injection of large amounts of NSILA-S leads to acute hypoglycaemia (Froesch et al. 1966; Oelz et al. 1972). Since most of the circulating NSILA-S is present in serum in a large molecular form (Zapf et al. 1975b) and since it has a half-life of around 4 h (Kaufmann et al. 1977) it appeared unlikely that NSILA-S was a major factor besides insulin in regulating glucose homoeostasis. On the other hand, Megyesi et al. (1975) have found that NSILA-S levels in man increase acutely after glucose administration and decrease again rapidly when normoglycaemia is reached. Fig. 2 shows NSILA-S levels in dogs after an intravenous glucose load of 1.5 g/kg body weight. As may be seen from Fig. 2 the variation in NSILA-S levels was considerable. However, there was no consistent increase that could be attributed to the glucose infusion.
Earlier, we have found that the concentration of NSILA-S in dogs decreases over a period of 4 to 6 days after pancreatectomy and that the levels were restored towards normal after a few days of insulin therapy (Froesch et al. 1975; Eigenmann et al., in press). Since prolonged hyperglycaemia and insulin deficiency are two major metabolic and endocrine changes associated with low

![Graph showing NSILA-S and glucose levels](image)

**Fig. 2.**
NSILA-S levels (●-●) and blood sugar levels (■-■) at zero time, 30, 60 and 120 min after the infusion of 1.5 g glucose/kg body weight. n = 5, mean ± se are given.

**Table 1A.**
NSILA-S and glucose values of 3 dogs, infused with 1.5 g glucose/kg body weight, after pre-infusion of 1.0 g mannoseptulose/kg body weight.

<table>
<thead>
<tr>
<th>Time intervals (h)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tbody>
<tr>
<td><strong>NSILA-S μU/ml serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>321 ± 4</td>
<td>341 ± 27</td>
<td>323 ± 8</td>
<td>310 ± 33</td>
<td>323 ± 38</td>
<td>314 ± 27</td>
<td></td>
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<tr>
<td><strong>Glucose mM</strong></td>
<td></td>
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<tr>
<td>3.82 ± 0.11</td>
<td>25.19 ± 3.16</td>
<td>22.86 ± 2.33</td>
<td>15.54 ± 2.44</td>
<td>10.37 ± 1.66</td>
<td>7.6 ± 0.61</td>
<td></td>
</tr>
</tbody>
</table>
**Table 1B.**

NSILA-S and glucose in 3 dogs after infusion of mannoheptulose (1.0 g/kg body weight).

<table>
<thead>
<tr>
<th>Time intervals (h)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSILA-S µU/ml serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>276 ± 109</td>
<td>223 ± 61</td>
<td>268 ± 105</td>
<td>252 ± 88</td>
<td>221 ± 71</td>
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<tr>
<td><strong>Glucose mM</strong></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>3.77 ± 0.32</td>
<td>5.99 ± 1.66</td>
<td>8.10 ± 2.71</td>
<td>10.6 ± 2.16</td>
<td>8.49 ± 0.27</td>
</tr>
</tbody>
</table>

Mean ± se are given.

**Fig. 3.**

NSILA-S levels (○—○), and blood glucose levels (■—■) at zero time, 5, 30 and 60 min after the injection of 0.25 U Actrapid/kg body weight in 4 dogs.

Mean ± se are given.
In order to test the acute influence of insulin-induced hypoglycaemia, 0.25 U of Actrapid/kg body weight was administered iv to 4 dogs. Despite a drastic fall in blood glucose levels (Fig. 3) the NSILA-S levels remained unchanged.

NSILA-S is significantly decreased in pituitary dwarfs and elevated in acromegalic patients (Schlumpf et al. 1976). We tried to clarify the question whether an acute elevation of growth hormone levels is followed by an immediate increase in NSILA-S concentrations. 0.2 mg of HGH/kg body weight was administered iv to 3 dogs. According to Pfeiffer et al. (1965) HGH is fully active in the dog. Fig. 4 shows the expected rise in free fatty acids following a dose of 0.85 to 1.5 mMol/l of serum. No significant changes in the NSILA-S levels were observed over a period of 2 h after growth hormone administration.
DISCUSSION

In order to study NSILA-S levels in individual sera one needs 1) a method for extracting NSILA-S from serum and 2) reproducible assay procedures. The one-step chromatographic procedure of Schlumpf et al. (1976) was found to be satisfactory and the two assay systems – bioassay and serum protein binding assay – were found to give reproducible results with a good correlation between the two assays.

Our results demonstrate that NSILA-S levels are not acutely influenced by the following metabolic manipulations 1) hyperglycaemia, acutely induced by the iv administration of glucose with or without blocking insulin release, 2) hyperglycaemia induced solely by blocking the insulin release by the iv administration of mannoheptulose, 3) hypoglycaemia brought about by the iv administration of insulin. Hence our results in dogs contrast sharply with those of Megyesi et al. (1975) obtained in humans. These investigators found elevated levels of NSILA-S after an oral glucose load and slightly decreased levels after insulin administration.

NSILA-S levels are decreased in pituitary dwarfs (Schlumpf et al. 1976) and in hypophysectomized dogs and rats (Froesch et al. 1976; Eigenmann et al. 1975). Schlumpf et al. (1976) did not find a clear-cut increase of the NSILA-S levels in 6 pituitary dwarfs under growth hormone therapy. These results are in keeping with our present negative findings in dogs in which no rise in NSILA-S after one single injection of HGH was found. Contrary, to our findings, Megyesi et al. (1975) described an acute rise in plasma NSILA-S following iv administration of HGH to man. The discrepancies between our results and those of Megyesi et al. (1975) regarding acute changes in NSILA-S levels, may be due to species variation. Moreover, Megyesi et al. (1975) used an assay for NSILA-S which is considerably different from our own assay procedures. In the radioreceptor assay with liver cell membranes (Megyesi et al. 1975) a NSILA-S preparation of approximately 10–15 % purity was iodinated whereas we have used 50–60 % pure NSILA for iodination.

The lack of acute changes in NSILA-S levels after metabolic and endocrine manipulations is in good agreement with the results of Kaufmann et al. (1977) according to which NSILA-S has a half-life in the rat of approximately 4 h. This long half-life of NSILA-S is explained by the fact that more than 95 % of total NSILA is present in serum in the bound form in which it may not be accessible for tissues. These results are also in keeping with the long held hypothesis according to which NSILA-S does not appear to be a major factor in glucose homoeostasis. In our laboratory (Froesch et al. 1976) it was demonstrated that NSILA-S acts via specific receptors of cells and tissues (Zapf et al. 1975a) concerned with growth in concentrations between 0.1 and 10 μU/ml. Concentrations of small molecular NSILA-S in serum are also of
this order of magnitude. In physiological terms NSILA-S thus appears, to be an insulin-like growth factor rather than a factor controlling glucose homoeostasis.

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REFERENCES


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