PRELIMINARY COMMUNICATION

Insulin and Insulin-Like Activity in Serum and Extravascular Fluid

By

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Summary. In fasted, fed or *i.v.* glucose injected rats, the close approximation between immuno-reactive insulin (IRI) and insulin-like activity (ILA) in the abdominal lymph, contrasts with the high discrepancy between IRI and ILA in the corresponding serum. Implications of the physiological role of IRI and ILA are discussed.

Résumé. Chez le rat, à jeun, en état post-prandial ou après injection intraveineuse de glucose, les taux d'insuline mesurés dans le sérum par la méthode immunologique (IRI) sont beaucoup plus élevés que les taux obtenus par les dosages biologiques (ILA) — Par contre, les valeurs d'IRI et d'ILA tendent à être équivalentes dans le

liquide extracellulaire (lymphe abdominale) — Le rôle physiologique de l'IRI et l'ILA est discuté.

Zusammenfassung. Bei nicht gefütterten und gefütterten Ratten und auch bei Ratten, denen Glukose *i. v.* injiziert worden war, zeigten sich in der abdominalen Lymphe fast gleiche Werte für das immunologisch bestimmbare Insulin (IRI) und die insulinähnliche Aktivität (ILA), was mit der großen Diskrepanz zwischen IRI und ILA des entsprechenden Serums im Gegensatz steht. Die physiologische Bedeutung der IRI und ILA werden diskutiert.

In general, absolute values for immuno-reactive insulin (IRI) of a particular serum¹ are smaller than the corresponding estimations of insulin-like activity levels (ILA) obtained with *in vitro* assays or *in vivo* intraperitoneal techniques^{1,6,9}. This discrepancy between values of ILA and IRI might be due to lack of specificity of the biological assays, or to serum factors with an insulin-like activity, or to forms of insulin in blood which do not react with insulin antibody.

Another possibility is that high values of ILA might have no physiological basis and might merely result from technical artefacts. *In vivo*, tissues are perfused through vascular membranes which limit the quantity and quality of extravascular proteins. In the biological assays, the surface and extravascular spaces of the isolated tissues are directly exposed to high concentrations of serum proteins normally maintained intravascularly.

To compare and contrast the "insulin" measurable by various assay systems in both the intravascular and extravascular spaces, samples of rat serum and lymph (which presumably approximates interstitial fluid) were simultaneously collected under various metabolic conditions.

Materials and Methods

A laparotomy was performed on normal male Sprague-Dawley rats, anesthetized with sodium pentobarbital, lymph was drained from the cysterna chyli, and blood was collected from the tail. Blood and lymph were allowed to clot at 4°C for four hours and were centrifuged; the supernatant fluids were collected and frozen. Similar amounts of serum and lymph from individual fed and fasted rats were pooled. The glucose was measured by the glucose-oxidase method² and IRI by a modified double antibody technique⁷. ILA was measured *in vitro* either by the conversion of glucose-1-¹⁴C to ¹⁴CO₂ by rat adipose tissue^{5,6} or by the incorporation of glucose-U-¹⁴C into glycogen by rat hemi-diaphragm¹ in these *in vitro* techniques, samples of serum and lymph were assayed at a dilution of 1:4. Finally, an *in vivo* bioassay was performed using the intraperitoneal technique of Rafaelsen, as modified in this laboratory¹.

Results

Table 1 shows the values for glucose concentration, ILA by adipose tissue and IRI of four pools of serum and lymph collected from fed animals. In

Table 1. Glucose, ILA and IRI in pools of serum and lymph collected from fed rats

Pool	Glucose mg. per 100 ml.		ILA μ U per ml.		IRI μ U per ml.	
	Serum	Lymph	Serum	Lymph	Serum	Lymph
A	230	220	200	50	36	23
B	190	210	190	55	47	42
C	185	205	450	90	65	50
D	165	200	272 $\pm$ 31*	140	45	38
	192	209	278	84	48	38

* Mean value and S.E.M. of four different assays.

lymph, low amounts of ILA were present, averaging 30% of that found in the corresponding serum. In contrast, IRI in lymph was approximately 80% of that measured in serum. A similar pattern was demonstrated, assaying individual samples of serum and lymph from 16 rats, either fed or fasted 48 hours⁴. The low level of ILA in the rat abdominal lymph was confirmed by the intraperitoneal assay technique. Table 2 summarizes values obtained by this method and the three other assay techniques, using pooled samples of serum and lymph from fed and fasted animals.

20 over-night fasted rats, after a rapid intravenous injection of 1 gm of glucose / kg of body weight. The accompanying Figure 1 shows that IRI increased promptly and similarly in both serum and lymph; the increase in serum ILA was striking, whereas it was questionable in the corresponding lymph.

To compare these changes to the more prolonged physiological alterations induced by a post-prandial state, individual rats were fed chow *ad libitum*, sacrificed at random, and five were selected for a high serum ILA, averaging $660 \pm 197 \mu\text{U/ml}$ (S.E.M.). The corresponding mean value of lymph ILA was 135 ± 47

Table 2. Comparison of different techniques for measurements of «insulin» in rat serum and lymph*

Pool	ILA		IRI		
	Intraperitoneal Assay		Adipose Tissue	Muscle	Immunoassay
	Muscle Glycogen	Adipose Glycogen	In Vitro	In Vitro	
Fed Serum	135	345	450	>250	65
Fed Lymph	< 20	< 20	90	40	50
Fed Serum	37	94	110	30	32
Fast Lymph	< 20	< 20	45	0	20

In order to exclude a factor inhibiting ILA in lymph, 200 $\mu\text{U/ml}$ of crystalline pork insulin were added to lymph. In other experiments, serum and lymph were mixed in a proportion of 1:1. In each case, the recovery of exogenous and endogenous ILA approximated 100%.

Changes in glucose, IRI and ILA concentrations were measured in serum and lymph pooled from

$\mu\text{U/ml}$. Serum and lymph IRI gave respective values of 63 ± 3 and $52 \pm 6 \mu\text{U/ml}$ and the mean glucose concentration in serum was 234 mg% and in lymph, 250 mg %. These data again suggest that elevation of serum ILA is not reflected in lymph.

Discussion

Serum ILA of normal rats in a variety of metabolic conditions cannot be recovered in similar concentrations in lymph, whereas serum and lymph IRI are very similar. The insulin measured by the immunoassay is a small molecule which quickly equilibrates between the intravascular and extravascular spaces, as it can be detected in rat lymph within a few minutes following an intravenous load of glucose. Current studies in rats injected intravenously with crystalline insulin or ¹³¹I-labeled crystalline insulin support the rapid diffusion of IRI into the lymphatic vessels in a pattern resembling the diffusion of inulin.

Insulin-like activity, over and above the measurable immunoreactive insulin, might be either the effect of a non-specific protein with insulin-like activity or of an insulin complex of high molecular weight. *In vivo*, these large molecules are mainly restricted to the vascular space by the capillary membranes, and thus are hardly detectable in the interstitial fluids. The low level of ILA in the rat abdominal lymph and in human spinal fluid⁹ or transudates⁸ supports this hypothesis. These data are also compatible with the possibility that ILA might be merely a technical artefact, encountered when the outer surface of isolated rat adipose tissue is exposed to high concentrations of serum proteins, a situation never achieved *in vivo*. The protein concentration in the rat abdominal lymph is 50% of the serum concentration and might partially

Fig. 1. Levels of serum and lymph glucose, ILA and IRI, before and during rapid i. v. glucose administration

* Values in $\mu\text{U/ml}$, corrected for dilution, except for muscle in vitro.

account for the low level of ILA. Thus, the physiological role of ILA seems questionable, and IRI appears to be the active metabolic principle *in vivo*.

Other evidence indirectly supports this concept. An increase of 40 μ U/ml of IRI in brachial arterial blood can induce a definite metabolic response in the forearm of man¹¹. Furthermore, a concentration of IRI as low as 20 μ U/ml can be detected by the intraperitoneal assay technique¹. In these two studies, the interstitial IRI has presumably been significantly enhanced and a metabolic effect demonstrated. Certainly, if the total physiological activity of ILA as measured *in vitro* were to be active on tissues *in vivo*, a small increment of added crystalline insulin of the order of 20 or 40 μ U/ml would not be so readily detected by a metabolic effect.

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