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Clearance Studies of Insulin and Nonsuppressible Insulin-Like Activity (NSILA) in the Rat Liver.* (32392)

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Two components of insulin-like activity (ILA) have been described in serum. One is that fraction readily identifiable by immunoassay(1); the other is that fraction of serum, nonreactive with insulin specific antiserum and, hence, "nonsuppressible" on bioassay(2,3,4). This latter component has been studied extensively by investigators using widely differing methods of preparation and assay(2,3,4). This fraction has been called "bound" insulin(4), "atypical" insulin(3) and nonsuppressible insulin-like activity (NSILA)(2,5). Recent investigations in our laboratory(6,7,8) support the initial postulate of Kipnis and Stein(9) that these forms of ILA are probably identical. Because of the lack of indisputable evidence readily identifying this component as insulin or one of its metabolites, we prefer the terminology of Froesch *et al*(2,5), nonsuppressible insulin-like activity (NSILA).

Studies of the quantitation of NSILA in diabetes mellitus and following oral glucose administration have yielded inconsistent and confusing results(2,3,10). In most instances, major changes in NSILA have not been demonstrated in humans. Previous work has failed to establish whether or not NSILA is cleared in the circulation(11). Therefore, these investigations were undertaken to determine whether the liver removes NSILA from the circulation and to compare its removal rate with that of crystalline insulin.

Methods. NSILA was prepared from pork plasma because of the lack of availability of sufficient human or rat plasma. When dialyzed and assayed directly at 2.5% concentration in the isolated fat cell bioassay(12, 13), pork plasma contained 60 μU NSILA/ml. After preparative chromatography on Dowex 50(4,8,13), the lyophilized NSILA preparation contained 250-300 μU/mg.

Intact livers weighing 14-15 g from male Sprague-Dawley rats (450 g), fed *ad libitum*, were perfused cyclically according to the method of Miller *et al*(14). Heparinized rat blood, diluted 1:3 with Ringer's solution (Hematocrit = 17%), was used; the total perfusate volume was 80 ml. Blood flow through the liver was maintained at 25-30 ml/minutes, and the perfusions terminated after 4 hours. Viability of the preparation was assessed by the appearance of the liver and bile output. Sampling was done from the hepatic effluent and not more than 10 ml (12%) were removed during any single perfusion.

After a one-hour control period, crystalline pork insulin (Lilly 25 U/mg) was added to the perfusate to give an initial concentration of 2000 μU/ml. Serial samples were taken throughout three hours. In additional experiments, 100 mg porcine NSILA (300 μU/mg) were introduced into the perfusion bath and serial sampling performed as above.

Samples from the 2 insulin perfusions and

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the 4 NSILA perfusions were dialyzed against 2 liters of Krebs-Ringer-bicarbonate buffer—10 mg % glucose, 4°C, at constant volume and measured by the isolated fat cell bioassay (12,13). The results are plotted as per cent of maximal (initial) concentration determined 5 minutes after the insulin or NSILA had been added to the bath, when complete mixing had occurred. From semi-logarithmic plots of this data, the half-lives of insulin and NSILA in this system were estimated. No correction was made for the error introduced by the decreasing perfusate volume, as this did not exceed 12%.

Results. The half-life of crystalline pork insulin in the rat perfusion system was determined to be 14 minutes as shown in Fig. 1A and 2A. In contrast to the short half-line of porcine insulin, the half-life for pork NSILA approximated 63 minutes (Fig. 1B and 2B).

The hepatic clearances (k) of the insulin and NSILA were also calculated from formula (A) of Mortimore and colleagues(15) and Bürgi *et al*(16):

$$(A) \qquad K = \frac{2.3 \times V}{\Delta t} \times \text{Log} \frac{Ca}{C_F}$$

where k = hepatic clearance or reaction-velocity constant

V = volume of perfusion fluid during sampling time Δ t.

Ca = insulin or NSILA concentration at beginning of the time interval Δ t.

C_f = insulin or NSILA concentration at end of the time interval Δ t.

In the present experiments, the k value for insulin was 3.59 ml/minute and for NSILA 0.58 ml/minute.

Similarly, the half-life of insulin and NSILA in the perfusion was calculated from formula (B) of Bürgi *et al.*

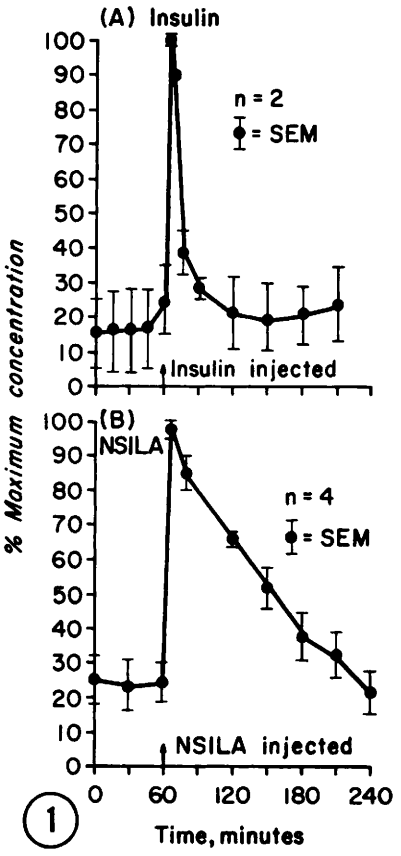
(B) $T_{1/2} = \text{Log } 2 \times 2.3 \times V/k$, where $T_{1/2}$ = half-life of insulin or NSILA. The calculated $T_{1/2}$ for insulin was 15 minutes and 67 minutes for NSILA which agree quite well with the graphic calculations (Fig. 1 and 2).

Discussion. The value of 14 minutes obtained for the half-life of insulin in the perfused liver is in accord with data in the literature(15). The k of 3.59 ml/minute for insulin is consistent with the values of 2.67 ml/minute and 3.05 ml/minute obtained by others(15,16). The higher flow rate through our perfusion system (25-30 ml/minute) may serve in part to explain differences in comparison to the data of Mortimore *et al*(15) and Bürgi *et al*(16) in which perfusion rates of 7-10 ml/minute were used.

Based on the assumption that hepatic inactivation is primarily responsible for clearance of these molecules in the intact rat, a theoretical T 1/2 for insulin and NSILA can be calculated using the approximation of Farris and Griffith(17) and formula (B). By this calculation, the half-life of insulin is similar to the value of 3.9 minutes calculated by Bürgi *et al*(16) and others(18) but is at variance with the actual insulin half-life values obtained by Goodner *et al* of 20 minutes(19). Because such calculations are not necessarily precise, it is probable that these half-lives in the intact animals represent only reasonable approximations. However, the relative clearances of insulin and NSILA are distinctly different.

It is of interest to speculate upon the significance of a prolonged half-life of NSILA. The half-life of NSILA (63 minutes) is 4 to 5 times that of crystalline insulin. The clearance of NSILA by the liver may well be related to uptake by the reticulo-endothelial system, rather than inactivation at a site of biological action. Moreover, the clearance of this material also supports the concept of a site of production within the organism, as serum levels have been found to remain relatively constant. Such a site of production, however, has not been ascertained(8). It is possible that the prolonged half-life of NSILA may explain in part the lack of acute changes in serum concentration of this material during physiologic experimentation.

Summary. In the isolated rat liver perfusion system, with a blood flow of 25-30 ml/minute, the biological half-life of crystalline pork insulin was determined to be 14 minutes and pork nonsuppressible insulin-like activity (NSILA) 63 minutes. The prolonged half-life of NSILA is consistent with the apparent lack of change in serum concentration in



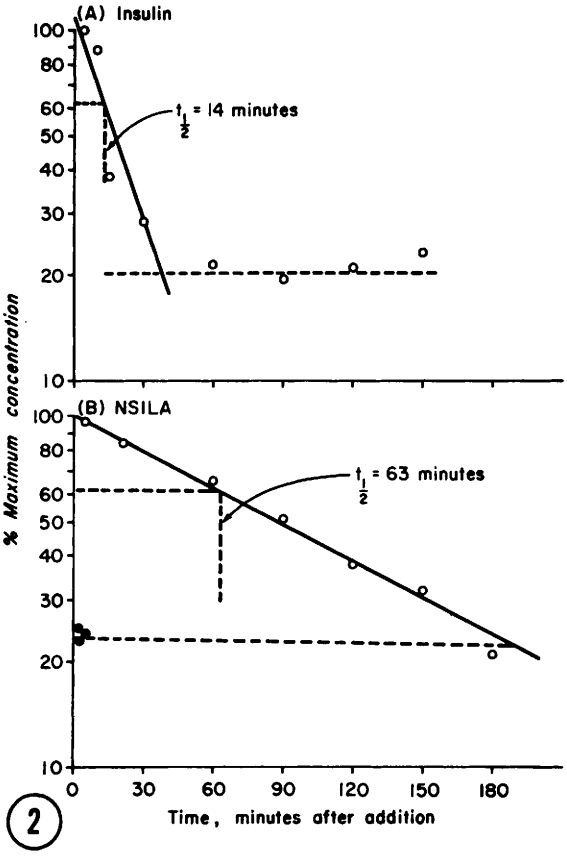


FIG. 1. Hepatic clearances of crystalline pork insulin (A) and pork NSILA (B) are plotted as the percentage of the T_{65} maximum (initial) concentration of insulin-like activity (ILA) determined by fat cell bioassay. The 0 to 60 minute period represents a control period, before addition of insulin or NSILA. Uptake during the control periods reflects the endogenous insulin and NSILA of diluted rat serum.

FIG. 2. Hepatic clearances of crystalline pork insulin (A) and pork NSILA (B) are shown on a semilogarithmic plot and the t 1/2 for each is indicated. In each instance the dotted line reflects the baseline activity of the diluted rat serum. Open dots are the sequential points for each experiment (A) and (B), where (A) — average, n = 2 and (B) — average, n = 4. Closed dots in (B) are 3 control points before addition of NSILA and help delineate the baseline activity in these experiments.

clinical situations and under physiologic experimentation.

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Induction of Mitochondrial α-Glycerophosphate Dehydrogenase by Thyroid Stimulating Hormone and Thyroid Hormone Analogues.* (32393)

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Y-P Lee *et al*(1) observed that administration of thyroid hormone to rats led to a marked increase in the activity of liver mitochondrial alpha-glycerophosphate dehydrogenase (LM-GPDH) but caused no change in the activity of the soluble diphosphopyridine nucleotide linked GPDH. Reports from this and other laboratories indicate that the induction of LM-GPDH by thyroid hormone may result from the acceleration of enzyme protein synthesis, which is mediated through an increase of RNA synthesis, as judged by the fact that this induction is sensitive to the inhibition by ethionine, puromycin, actinomycin D, 5-fluorouracil (1-5) and fasting(5, 6). One of the characteristic features of thyroid hormone action is its biphasic effect on the body growth as well as the metabolism of some cellular constituents(7). Since the dosage of thyroid hormone used to observe the induction of LM-GPDH was much greater than the amount of thyroid hormone normally produced under physiological conditions, it is important to determine whether the observed induction is a physiological or a pharmacological effect.

In this communication, experimental results are presented which further support the view that the LM-GPDH activity is under the control of thyroid hormone in that administration of either L-triiodothyronine or thyroid-stimulating hormone (TSH) to hypophysectomized rats resulted in enzyme induction and a good correlation was found between the inducing ability of iodine containing structurally similar compounds and its hormonal activity.

Materials and methods. All animals utilized in these studies weighed 200-280 g (intact) or thyroidectomized (THX) and hypophysectomized (HYX) rats weighed 100-130 g and were obtained from the Charles River Laboratories. They were fed *ad libitum* on a Purina chow-diet and were allowed free access to drinking water. For maintenance of THX rats 1% calcium gluconate was added in the drinking water. The THX and HYX rats were not used until 4 weeks after their receipt from the supplier. During this period, the HYX rats gained essentially no weight.

3,3′,5-triiodo-L-thyronine (T_3), 3, 3′, 5,5′-tetraiodo-L-thyronine (L-thyroxine), 3, 3′, 5,5′-tetraiodo-D-thyronine (D-thyroxine), 3-iodo-L-thyronine and 3-iodo-L-tyrosine were purchased from Sigma Chemical Co. 3,5-diiodo-L-thyronine was obtained from Mann Research Laboratories. 3,5-diiodo-L-tyrosine was obtained from Nutritional Biochemicals

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