Hepatic Production of an Albumin-Associated Insulin Inhibitor* (33625)

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It has been proposed by Vallance-Owen *et al.* (1) that the insulin inhibitor associated with human plasma albumin may be the B-chain of insulin. At the present time, there is no direct evidence to support this. However, patients injected with exogenous insulin or intravenous tolbutamide show increased insulin inhibitory activity after injection (2). These observations suggest the possibility that the insulin molecule *per se* may be involved in production of the insulin inhibitor, possibly as a breakdown product. Since the liver is the major site of insulin breakdown, the effect of perfusing the isolated liver with normal rat serum, with and without added insulin, was studied to determine if increased amounts of insulin inhibitor are produced. In addition, inhibitory effects of albumin obtained from hypophysectomized and pancreatectomized rats were studied in order to determine whether low circulating insulin levels affect the degree of insulin inhibitor present in albumin.

Materials and Methods. All rats used were nonfasted males unless otherwise indicated. Sprague-Dawley rats, weighing 200–400 gm, were used as donors for preparation of control rat albumin. Hypophysectomized rats were obtained from Hormone Assay Laboratory, Inc., Chicago, Illinois. They were received 10–14 days after operation and weighed 150–200 gm. Total surgical pancreatectomy was performed on Sprague-Dawley rats weighing 100–130 gm (3). These animals were then maintained on subcutaneous saline injections and standard laboratory chow and sacrificed 48 hr after pancreatectomy. In all cases the rats were anesthetized with Nembutal and blood was drawn from the abdominal aorta using a heparinized syringe. Albumin was extracted from the pooled serum using Debro's method as modified by Vallance-Owen (4).

Liver Perfusion. The liver perfusion system used has been described in detail by Penhos (5). Livers from 400–500-gm rats were perfused with 50–60 ml of heparinized normal rat blood or serum. The pool of blood or serum was divided into three equal parts (I, II, III). Part I was kept at room temperature for the duration of the perfusion and was not perfused through liver. Part II was perfused through liver and III was perfused through liver with the addition of insulin. Two perfusions were run simultaneously. At the end of a 30-min equilibration period, 1 ml of saline was added to perfusion II and 1 U insulin in 1 ml of saline was added to perfusion III. The perfusions continued for 30 min longer. At the end of the perfusions, samples I, II, and III were collected and albumin was extracted from each. Serum was used in most perfusions in order to eliminate hemolysis seen in certain of the whole blood perfusions. Experimental results were the same whether whole blood or serum was used.

Diaphragm Assay. Albumin preparations were tested for insulin inhibitory activity by incubating with paired cut hemidiaphragms obtained from 140–170-gm rats fasted overnight. The incubation medium was Krebs-Henseleit-bicarbonate buffer containing 250 mg/100 ml glucose–200 mg gelatin and gassed with 95% O_2–5% CO_2. Paired hemidiaphragms were incubated, in triplicate, with and without 2% or 4% albumin and with and without 1000 μU insulin/ml. Glucose was determined by the method of Somogyi-Nelson (6) and expressed as milligrams glucose uptake/gram wet weight tissue/80 min. The glucose uptake of the hemidiaphragm incubated in buffer plus insulin was compared with the

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paired hemidiaphragm in albumin plus insulin. *P* values for the difference between these were computed by a paired *t* test.

Results. The experimental results may be seen in Table I. At a concentratiin of 2% normal rat albumin (control Part I) had no significant effect upon insulin action. On the other hand, 4% albumin (control) impaired the action of insulin. Albumin obtained from normal rat serum which was pumped through the liver perfusion system in the absence of liver, when tested at 2% concentrations did not result in any impairment of insulin action. However, baseline stimulation of glucose uptake was apparent (+ 1.12 mg/gm). Albumin obtained after liver perfusion significantly altered the insulin action. This was more prominent in the samples which were perfused with insulin. Baseline stimulation was also observed with these perfused samples (+ 0.93 mg/gm and + 1.2 mg/gm respectively).

Hypophysectomy or pancreatectomy had no obvious effect upon the capacity of 4% albumin to inhibit the action of insulin.

Discussion. The data obtained suggests that rat albumin behaves in a fashion similar to human albumin in that no significant insulin inhibition occurs at 2% concentrations while at 4%, insulin inhibition is observed (7). Perfusion of normal rat blood or serum through rat liver results in the appearance of insulin inhibitor in the extracted albumin. This can be demonstrated at 2% albumin concentrations. Those samples perfused through liver in the presence of added insulin appear to be more inhibitory than those perfused without insulin.

Several problems enter into the quantification of inhibitor levels. When an albumin preparation produces a baseline stimulation of glucose uptake, or has insulin-like action (ILA), calculation of the percentage of inhibition of the insulin effect in albumin compared with the control insulin effect is not meaningful. (The insulin effect is measured as the increment in glucose uptake in insulin over the baseline uptake.) In our hands baseline stimulation by albumin preparations has occurred only rarely and is poorly understood

TABLE I. Experimental Results.

Albumin	No. samples[a]	Concentration tested	Glucose uptake (mg/g/80") A[b]	B	AI	BI	BI—AI	*p* for AI vs BI
Control (Part I)	6	2%	4.99 ± .42[d]	4.93 ± .50	7.94 ± .26	8.54 ± .35	+0.60	n.s.
Control perfused plus liver (Part II)	5	2%	5.27 ± .73	4.34 ± .64	7.34 ± .48	9.07 ± .64	+1.73	<.005
Control perfused plus liver plus insulin (Part III)	5	2%	4.75 ± .45	3.55 ± .94	6.52 ± .45	8.89 ± .63	+2.37	<.001
Control perfused without liver	1[c]	2%	4.71 ± .37	3.59 ± .12	7.67 ± .56	8.15 ± .57	+0.48	—
Control	1	4%	4.69 ± .41	4.06 ± .33	5.97 ± .15	8.22 ±1.21	+2.25	—
Hypophysectomized	3	4%	5.55 ± .92	5.27 ± .59	8.54 ± .26	10.96 ± .37	+2.42	<.01
Pancreatectomized	1	4%	2.38 ± .17	2.66 ± .23	4.48 ± .48	7.54 ± .55	+3.06	—

[a] Each sample represents the pool of approximately 6 rats, tested in triplicate.
[b] A = albumin, B = buffer, AI or BI = albumin or buffer + 1000 μU/ml insulin.
[c] *p* values could not be accurately determined with a single sample.
[d] SEM.

(8, 9). However, comparison of the differences between glucose uptake in the buffer plus insulin vs albumin plus insulin is possible. The albumin samples obtained from serum perfused through the liver perfusion apparatus all showed stimulated baselines. Despite roughly equivalent ILA, the three samples behaved differently. The sample which was perfused without liver had no effect on insulin action. The other two samples showed significant insulin inhibitory action. Thus one can conclude that the liver in some way increases insulin inhibitor associated with albumin. The addition of insulin seems to have further increased inhibitor levels.

The observations in hypophysectomized animals and pancreatectomized rats indicate that inhibitor activity persists despite lack of many diabetogenic hormones in the former and an obviously decreased insulin level in the latter. Since the t_{50} of the inhibitor is unknown it is not possible to conclude that absence of insulin or diabetogenic hormones has no influence upon inhibitor levels.

At the present time, the status of the insulin inhibitor associated with albumin remains confused. The data obtained here add the possibility of a hepatic source for the inhibitor but are somewhat less than definitive in view of the stimulated baselines observed with the perfused samples. The role of insulin as a precursor of the inhibitor is still likely. Interpretation of the persistence of inhibitor in pancreatectomized rats does not rule out an insulin-precursor hypothesis since the duration of pancreatectomy was only 48 hr.

Summary. The nature of the insulin inhibitor associated with plasma albumin remains an enigma with possibilities ranging from the B chain of insulin to artifacts due to dialysis membranes or trichloracetic acid. Our observations that exogenous insulin or tolbutamide cause an increase in inhibitor levels in normal subjects suggested that insulin degradation (probably hepatic) could be involved (2). To test this possibility, normal rat serum was perfused through rat liver with and without added insulin. Subsequently atbumin was isolated and insulin inhibitory activity was measured. Inhibitor activity was significantly increased by liver perfusion. An apparent further increase occurred with added insulin.

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