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SHORT COMMUNICATION

Rat Renal Glomeruli and Tubules have Specific Insulin Receptors of Differing Affinity

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SUMMARY

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Purified isolated rat renal glomeruli and tubules were incubated with [^{125}I]insulin and the binding and degradation of the hormone were followed. Both glomeruli and tubules showed specific binding of [^{125}I]insulin, which reached a plateau between 40 and 90 min of incubation. In the presence of increasing concentrations of unlabeled insulin ranging from 5-50,000 ng/ml, binding of [^{125}I]insulin to the glomerular receptor(s) exhibited a higher affinity than that to the tubular receptor(s). Scatchard plots of the binding data were curvilinear, consistent with two or more classes of receptor sites in each preparation or negative cooperative site-site interactions. These plots supported a higher affinity for glomerular receptor(s) and a higher binding capacity for tubular receptor(s). Insulin analogues were less potent than insulin itself in inhibiting the binding of [^{125}I]insulin to both glomeruli and tubules, and some were more effective in glomeruli than in tubules, again supporting the presence of receptor populations of differing affinity in the two preparations. Dissociation of bound [^{125}I]insulin from the tubular fraction was more rapid than that from glomeruli also supporting a lower affinity of the receptors for insulin in this preparation.

The kidney is a major site of pathologic complications in diabetes mellitus, particularly the microangiopathy affecting the glomerulus (1). The presence of specific insulin receptors in preparations of renal cell membranes from dog (2) and rat (3) has recently been described, but very little is known about the localization and characteristics of these receptors in the nephron (4). Such information is of particular interest in view of the reports that insulin reverses enzymatic anomalies seen in experimental diabetes such as increased glucosyl transferase activity in renal cortex (5), increased lysyl hydroxylase activity in isolated glomeruli (6) and decreased renal β-N-acetylglucosaminidase activity (7), all of which may be related to the accumulation of the collagenous-glycoprotein basement membrane extracellular matrix seen in this disease. Insulin has also been implicated in the regulation of renal electrolyte excretion

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(8, 9) and may be involved in the sodium retention that sometimes accompanies treatment of insulin-dependent diabetics (10). We have, therefore, investigated the binding and affinity characteristics of [^{125}I]insulin in isolated purified preparations of rat renal glomeruli and tubules.

Glomeruli and tubules were isolated concurrently from the renal cortex of 300–400 g male Wistar rats as described previously (11, 12), by a procedure employing magnetic iron oxide, which yields preparations of 99% purity without enzymatic treatment. The time course of [^{125}I]insulin binding to glomeruli and tubules was followed by incubating suspensions of the renal subfractions (glomerular protein range: 0.28–0.85 mg/ml; tubular protein range: 0.27–1.85 mg/ml) in Krebs-Ringer bicarbonate-1.5% bovine serum albumin buffer (pH 7.4) containing 1 ng/ml of [^{125}I]insulin (13) (specific activity 200–220 μCi/μg) at 22° with shaking, and removing 0.25 ml aliquots of the suspensions in triplicate at different time points for the measurement of bound [^{125}I]insulin. Glomeruli and tubules were rapidly separated from the incubation medium by centrifugation of the aliquots through 0.5 ml of a 1:2 mixture of dinonylphthalate:dibutylphthalate oil in a Beckman microfuge. After centrifugation the incubation medium containing unbound [^{125}I]insulin was separated from the pelleted renal fractions containing bound [^{125}I]insulin by the intervening oil layer. An aliquot (0.25 ml) of the upper aqueous layer was transferred by pipette into an equal volume of cold 20% trichloroacetic acid,[2] and insulin degradation measured in the incubation medium was estimated by measuring the proportion of trichloroacetic acid-soluble radioactivity. The remaining incubation medium and oil layer were aspirated off and the tissue pellets, after excision of the tube bottoms, were counted for radioactivity and then solubilized in 1 N NaOH for protein determination by the Lowry method (14). Nonspecific binding, defined as that measured in the presence of 50 μg/ml unlabeled insulin in the medium was determined in all experiments and subtracted from the total binding to yield specific binding. Nonspecific binding accounted for 20 to 25% and 30 to 35% of total binding for glomeruli and tubules, respectively. All binding data were corrected for insulin degradation by taking the radioactivity in the TCA-soluble fraction to represent degraded insulin ([^{125}I]insulin not exposed to tissue was greater than 97% precipitable by TCA). The binding data, corrected for nonspecific binding and degradation, was determined using the following expression:

$$\frac{\text{Specific Bound}}{\text{Total}}\ (\text{corrected}) = \frac{\text{Total Bound} - \text{Nonspecific Bound}}{\text{Total} \times \text{fraction intact}}$$

as described by Kahn *et al.* (15). Insulin degradation ranged from 2 to 13% in glomeruli and 6 to 53% in tubules after 90 min at 22°.

Specific binding of [^{125}I]insulin to both glomerular and tubular fractions occurred rapidly at 22° and reached a plateau between 40 and 90 min of incubation (Fig. 1), similar to the binding observed in isolated renal plasma membrane preparations (2, 3). Total [^{125}I]insulin binding at 90 min was 6.6% of the total intact insulin present for glomeruli (10.8 fmoles insulin/mg protein) and 3.6% for tubules (5.9 fmoles insulin/mg protein), adjusted to a protein concentration of 1 mg/ml renal subfraction. Both total and nonspecific binding are linear with protein concentration in the range used in these experiments. When the binding of [^{125}I]insulin (1 ng/ml) was allowed to reach a plateau (75 min at 22°) in the presence of increasing concentrations of unlabeled insulin ranging from 5–50,000 ng/ml, a continuous decrease in the percent bound compared to that bound in the presence of tracer alone was observed in both glomeruli and tubules (Fig. 2). At all concentrations of unlabeled insulin, there was a greater inhibition of [^{125}I]insulin binding in glomeruli than in tubules, indicating that these two renal subfractions contain insulin receptors that differ in their apparent affinity for the hormone. While 50% inhibition of [^{125}I]insulin binding in the presence of tracer alone was observed in glomeruli at a

[2] The abbreviations used are: TCA, trichloroacetic acid.

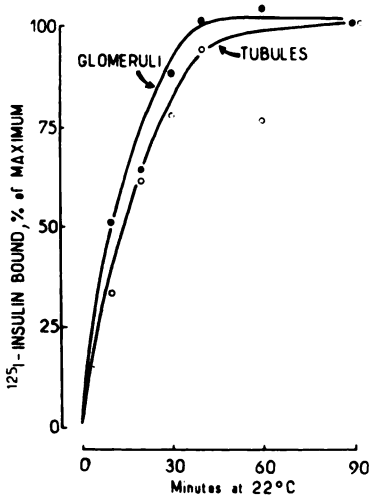

FIG. 1. *Time course of specific [^{125}I]insulin binding to isolated rat renal glomeruli and tubules*

Glomeruli and tubules isolated from the kidneys of three rats were suspended in 12 ml of buffer and incubated at 22° with 1 ng/ml [^{125}I]insulin in the presence and absence of 50 μg/ml unlabeled insulin. Aliquots in triplicate were removed at the indicated times for determinations of bound [^{125}I]insulin. All data have been corrected for nonspecific binding (22.9% of total [^{125}I]insulin binding for glomeruli and 32.3% for tubules at 90 min) and degradation (4.4% of total [^{125}I]insulin for glomeruli and 5.9% for tubules at 90 min) measured at each time point.

concentration of 2.8 nM unlabeled insulin, a ten-fold higher concentration of insulin (35 nM) was required to achieve a comparable inhibition of binding in tubules.

Scatchard analysis of the [^{125}I]insulin binding data to glomeruli and tubules (Fig. 3) yielded curvilinear plots consistent with two or more classes of receptor sites in each preparation, negative cooperative site-site interactions (16) or both. Quantitative interpretation of this data is difficult because we are dealing with organ subfractions, one of which—the glomerulus—is a multicomponent system containing three different cell types. However, the data are consistent with an overall higher affinity of the glomerular receptor(s) and an overall greater binding capacity of the tubular receptor(s). Whether these observed differences are due to unique receptor(s) in each preparation, differences in the type or extent of site-site interactions or differences in the relative contribution to binding of identical receptor class(es) present in both preparations cannot be determined at present.

The specificity of the insulin binding to both glomeruli and tubules and further indications for the different binding characteristics of their receptors were obtained by

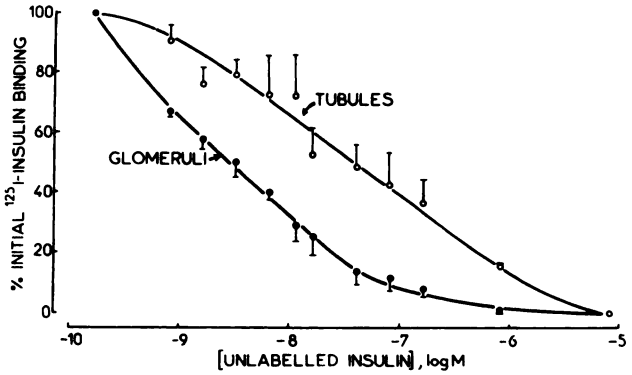

FIG. 2. *Comparison of inhibition of [^{125}I]insulin binding to isolated glomeruli and tubules by unlabeled insulin*

Glomeruli and tubules were incubated for 75 min at 22° in 1.5 ml microfuge tubes in a total volume of 1.06 ml containing 1 ng/ml [^{125}I]insulin and 0–50,000 ng/ml unlabeled insulin. At the completion of the incubation, triplicate 0.25 ml aliquots were removed for measurement of bound [^{125}I]insulin and the determination of insulin degradation. Initial [^{125}I]insulin binding represents the binding of [^{125}I]insulin measured in the absence of unlabeled insulin. All data are corrected for nonspecific binding and degradation and represent the means ± standard errors at each time point of 2–4 experiments using renal preparations derived from 3–4 animals per experiment. At the insulin concentrations examined the glomerular values differ from those of tubules in Student's *t*-test at the following probability levels for statistical significance: $p < 0.001$ (5 μg/ml), $p < 0.01$ (5, 20 and 250 ng/ml), $p < 0.05$ (10 and 70 ng/ml), $p < 0.1$ (40, 100, 500 and 1000 ng/ml).

comparing the ability of analogues of insulin to inhibit [^{125}I]insulin binding with that of insulin itself (Table 1). Insulin was more potent than any of the analogues studied in inhibiting [^{125}I]insulin binding to both glomeruli and tubules, with a greater effectiveness in glomeruli than in tubules, consistent with the higher affinity of the receptors for the hormone in the glomerular preparation. Insulin analogues with lower biological potency than insulin itself (17–20) were less potent than the native hormone in competing with [^{125}I]insulin for the binding sites, indicating the specificity of the receptors for the hormone in both preparations. Two of the analogues, proinsulin and the derivative with a blocked amino-terminal glycine in the A-chain (Al-Boc-Gly-insulin), discriminated to a certain degree in their inhibitory potency between glomeruli and tubules at high peptide concentrations (1 and 10 μg/ml), like insulin being more effective in glomeruli than in tubules. Two other insulin derivatives, despentapeptide insulin (a derivative with the last five carboxy-terminal B-chain residues missing) and that with an A_1-B_1 intra-molecular adipoyl crosslink (20), showed similar inhibitory effectiveness in both glomeruli and tubules. The selective inhibitory properties of proinsulin and to a lesser degree A_1-Boc-Gly-insulin, although not as marked in their discrimination as insulin itself, support the presence of different binding characteristics in glomerular and tubular insulin receptors. The ability of the analogues to inhibit [^{125}I]insulin binding in both preparations paralleled that observed previously in other preparations such as liver plasma mem-

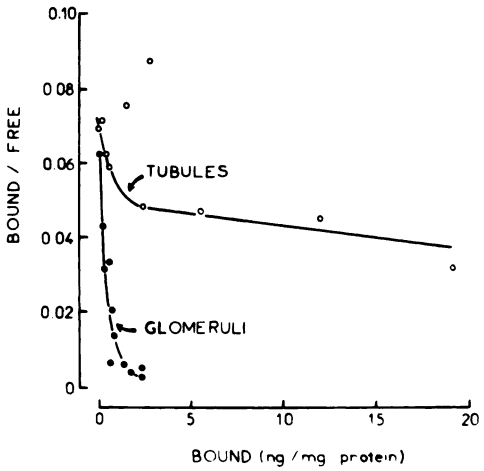

FIG. 3. *Scatchard analysis of data from a typical competition experiment in Fig. 2, plotting bound/free hormone versus hormone bound*

Experimental conditions and corrections for nonspecific binding and degradation are as described in the legend to Fig. 2 and in the text.

TABLE 1

Comparison of inhibition of [^{125}I]insulin binding to isolated glomeruli and tubules by unlabeled insulin and analogues

Experimental conditions were identical to those in Fig. 2. All values are the means ± standard errors of two experiments. $A_1 - B_1$ crosslinked insulin is a derivative with a 20-atom adipoyl bridge, referred to as (Z-lys) 2 (Ad) in ref. 20. The molar concentrations of the peptides equivalent to 1 μg/ml are insulin, A_1-Boc-Gly-insulin and $A_1 - B_1$ crosslinked insulin: 1.64×10^{-7} M, proinsulin: 1.05×10^{-7} M, and despentapeptide insulin: 1.74×10^{-7} M.

Peptide	% initial [^{125}I]insulin binding					
	0.1 μg/ml peptide		1.0 μg/ml peptide		10.0 μg/ml peptide	
	Glomeruli	Tubules	Glomeruli	Tubules	Glomeruli	Tubules
Insulin	25.5 ± 6.5	52.4 ± 9.2*	8.3 ± 2.9	36.6 ± 7.7*	—	—
Proinsulin	99.8 ± 14.2[b]	97.6 ± 4.2[b]	50.0 ± 4.8[b]	66.9 ± 7.1	11.7 ± 1.6	26.2 ± 4.2*
Despentapeptide insulin	104.2 ± 13.7[a]	99.6 ± 5.6[b]	29.5 ± 4.9[c]	26.7 ± 1.1	14.9 ± 8.0	9.1 ± 1.2
A_1-Boc-Gly-insulin	85.1 ± 20.6[b]	93.4 ± 9.5[c]	17.6 ± 0.5[c]	35.0 ± 0.8**	6.2 ± 1.2	8.8 ± 2.5
A_1-B_1 cross-linked insulin	—	—	82.2 ± 9.5[b]	81.2 ± 11.3[c]	42.6 ± 1.3	46.6 ± 3.4

The analogue values differ from those of insulin by Student's *t*-test at the following probability levels for statistical significance: [a] $p < 0.01$; [b] $p < 0.05$; [c] $p < 0.1$.

The glomerular values differ from those of tubules in Student's *t*-test at the following probability levels for statistical significance: ** $p < 0.05$; * $p < 0.1$.

branes (17, 19), indicating that the renal receptors were qualitatively similar in their binding characteristics to insulin receptors in other tissues.

The affinity of the glomerular and tubular receptors for insulin was further investigated by examining the dissociation of bound [^{125}I]insulin from the preparations upon dilution into a large volume of insulin-free medium. The results of a typical experiment show a rapid dissociation of bound [^{125}I]insulin from both glomeruli and tubules (Fig. 4), with that from the tubular fraction being more rapid. This supports a lower apparent affinity of the receptors for insulin in this preparation as compared to glomeruli. The half-time of dissociation of specifically bound [^{125}I]insulin from the glomerular receptors was approximately 42 min while that from the tubular receptors was only about four minutes, yielding dissociation rate constants of 1.7×10^{-2} min^{-1} and 1.7×10^{-1} min^{-1}, respectively.

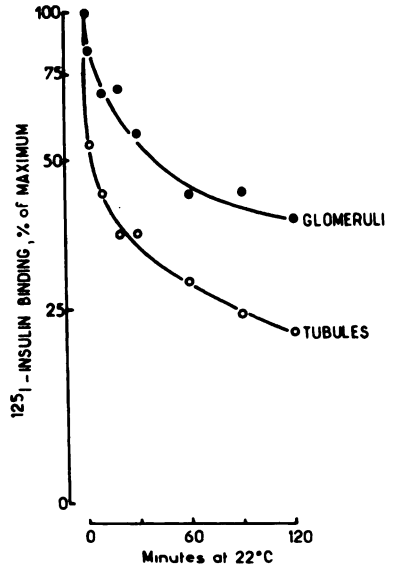

FIG. 4. *Time course of dissociation of specifically bound [^{125}I]insulin from isolated rat renal glomeruli and tubules*

Glomeruli and tubules isolated from the kidneys of five rats were incubated at 22° for 75 min in 7 ml of medium containing 1 ng/ml [^{125}I]insulin with and without the addition of 50 μg/ml unlabeled insulin for the determination of total and nonspecific binding. After removal of triplicate aliquots for measurement of binding at the plateau, the medium was removed by centrifugation and replaced with an equal volume of ice cold medium. Aliquots of 100 μl were then quickly dispensed into tubes containing 7.5 ml of medium at 22° and duplicate tubes centrifuged at the indicated times to promptly separate the medium from the pellet. The medium was then aspirated off and the pellets counted to determine remaining bound radioactivity. Nonspecific binding has been subtracted from the data at each time point, but no correction has been made for degradation.

Isolated rat renal glomeruli and tubules, therefore, contain specific insulin receptors which differ in their apparent affinity toward insulin and some of its analogues as demonstrated by the results of competitive binding experiments (Figs. 2 and 3, and Table 1) and experiments to measure the rate of dissociation of bound hormone (Fig. 4). The different binding characteristics of the preparations are maintained after correction for nonspecific binding and degradation of [^{125}I]insulin, and are indicative of different receptor populations in the two renal fractions and/or differences in the environment of the receptors. The differing affinity of the receptors for insulin in glomeruli and tubules is intriguing in light of the selective pathology of the specific renal lesions in diabetes mellitus that affect the glomeruli (21) to a much greater degree than the tubules. The basis for this differing affinity and its relation if any to the pathogenesis of the nephropathy in diabetes mellitus awaits further investigation.

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