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HIGH AFFINITY BINDING SITES FOR EPIDERMAL GROWTH FACTOR (EGF) IN RENAL MEMBRANES

Elizabeth Sack and Zvi Talor

Department of Medicine, Section of Nephrology
University of Illinois Hospital
and
West Side Veterans Administration Medical Center
Chicago, Illinois 60612

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The kidney produces large quantities of EGF but the role of the kidney in binding and degradation of EGF is unknown. We studied ^{125}I-EGF binding and degradation by highly purified cortical luminal and cortical basolateral membranes of rabbit renal cortex, and by medullary plasma membranes. Specific binding for ^{125}I-EGF was found for the first time in cortical basolateral and medullary plasma membranes (60-80% of total binding) but not in cortical luminal membranes. There was little degradation (less than 4%) of EGF by any of the membranes. Scatchard analysis of ^{125}I-EGF binding by cortical basolateral membranes revealed two distinct classes of binding sites: high and low affinity. The existence of high specific binding sites in cortical basolateral and in medullary plasma membranes suggests a physiologic role of EGF in the kidney.

Epidermal growth factor is a 53 amino acid peptide which has been shown to have physiologic importance in a variety of cell types. EGF is found in high concentrations in the urine and represents predominantly renal production rather than renal clearance of circulating EGF (1). High concentrations of prepro EGF have been localized to the thick ascending limb of Henle and to the early distal

Address correspondence to: Z. Talor, M.D., Section of Nephrology (M/C 793), University of Illinois Hospital, 840 South Wood Street, Chicago, Illinois 60612

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convoluted tubules of the kidney, but none was detected in the proximal tubule or in the collecting duct (2). The role of the kidney in binding and degradation of EGF has not been examined in detail although high affinity binding sites for EGF have been identified in cultured proximal tubule cells (3). These studies have not addressed the specific membrane localization (luminal vs. basolateral) of the binding sites nor have they examined other possible sites of binding and degradation. Therefore, we characterized EGF binding and degradation in highly purified rabbit renal cortical basolateral and luminal membranes. In addition, we examined binding and degradation of EGF in purified renal medullary membranes.

Membrane Preparation - Purified renal cortical luminal and basolateral membrane vesicles were prepared from cortical homogenates of New Zealand rabbit kidneys utilizing the method by Kinsella et al. (4) with mild modifications as previously described (5). Medullary plasma membranes were prepared from rabbit medullary homogenates as previously described (6). In each preparation, marker enzymes were determined in both homogenate and purified membranes. The marker enzyme activities were determined as previously described (5,6). Cross-contamination was calculated as follows: percent contamination of membrane A by membrane B = activity of marker enzyme p in A x 100/activity of marker b in B; where A and B are the respective membranes, and a and b the corresponding marker enzyme activities.

^{125}I-EGF Degradation - Degradation of ^{125}I-EGF was performed utilizing the TCA precipitation technique as previously described (7). In brief, 100 µg protein of membrane vesicles were incubated in a Krebs-Ringer phosphate buffer pH 7.5 containing 1% BSA in the presence of 2.5×10^{-10} M ^{125}I-EGF at 37° C. After different time intervals, an equal volume of ice-cold 20% TCA was added to 500 µl of the supernatant from the incubation mixtures, and following centrifugation (10,000 RPM, 10 min, Sorvall RC B5) the radioactivity was determined in the pellet and in the supernatant. The radioactivity in the supernatant, expressed as percent of total counts, represented ^{125}I-EGF degradation.

^{125}I-EGF Binding - For ^{125}I-EGF binding experiments, 100 µg protein of purified cortical luminal or basolateral membranes, or medullary plasma membranes were incubated at 22° C in a Krebs-Ringer phosphate buffer, pH 7.5, containing 1% BSA in the presence of 50 µl of ^{125}I-EGF (2.5×10^{-10} M) and in the presence and absence of 1×10^{-6} M unlabeled EGF. After different time intervals the reaction was stopped by addition of ice cold Krebs-Ringer-phosphate buffer (0.5 ml), pH 7.5 with Tris. Separation of bound from free EGF was performed by rapid centrifugation in Beckmann micro-ultracentrifuge tubes (35,000 g, 5 min). The supernatant was removed and the resulting pellet was washed with buffer and centrifuged again (35,000 g, 5 min). Each experiment was performed in duplicate, and appropriate blanks were used to calculate the binding of ^{125}I-EGF to centrifuge tubes in the absence of membranes. The final pellet was counted in a Beckmann gamma counter. Scatchard analysis of binding was done by varying the concentration of unlabeled EGF from 0 to 1×10^{-6} M. For multiple binding sites, kinetics of binding were determined by linear regression analysis of high and low affinity binding sites.

RESULTS AND DISCUSSION

Binding and degradation of ^{125}I-EGF was assessed in purified renal cortical luminal and basolateral, and in renal medullary plasma membranes. Cortical basolateral and luminal membranes were enriched more than 10 fold (compared to the homogenate) in Na-K-ATPase and alkaline phosphatase, respectively. The medullary plasma membranes were harvested at the 25-40% sucrose gradient interface. This fraction represents a mixture of medullary luminal (as evidenced by an enrichment in H^+-ATPase) and basolateral membranes (as evidenced by an almost 8 fold enrichment of Na-K-ATPase as compared to the homogenate).

Figure 1 shows the time course of ^{125}I-EGF degradation in cortical basolateral and luminal membranes, and in medullary plasma membranes and demonstrates less than 4% degradation of ^{125}I-EGF in any of the membrane preparations. These results indicate a low rate of EGF degradation by renal membranes in contrast to other peptide hormones of similar molecular weight (e.g. insulin). This lack of degradation may be important if EGF is to be transported from the basal to apical membrane, as has been recently demonstrated in Madin-Darby Canine Kidney cells (8). Since there was no significant degradation, the binding experiments were done in the absence of protease inhibitors.

Figure 2 (left panel) shows the time course of EGF binding to renal cortical

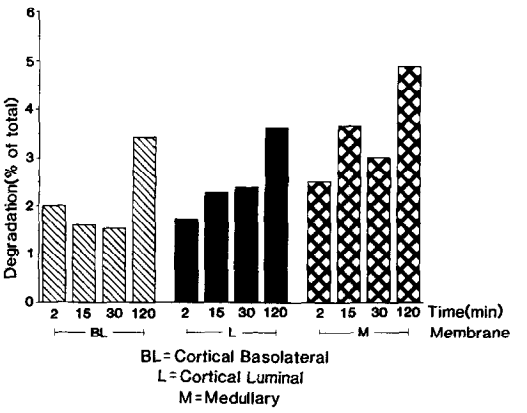


Fig. 1 **Time course of ^{125}I-EGF degradation**: An equal volume of ice-cold 20% TCA was added to 500 µl of the incubation mixture (at 37° C). The reaction was stopped after different time intervals.

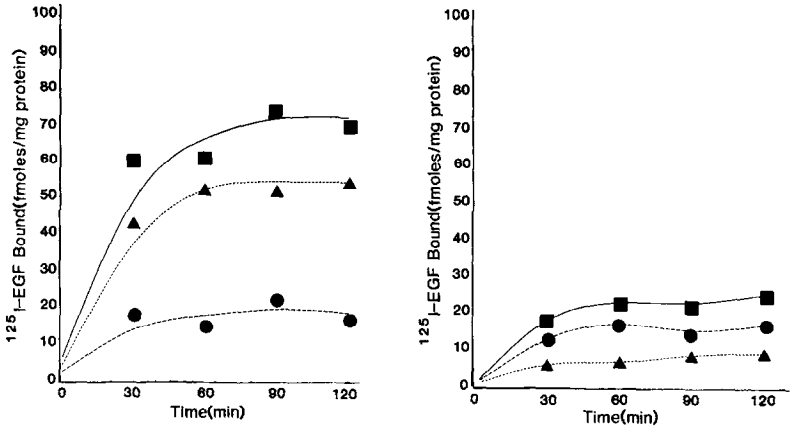


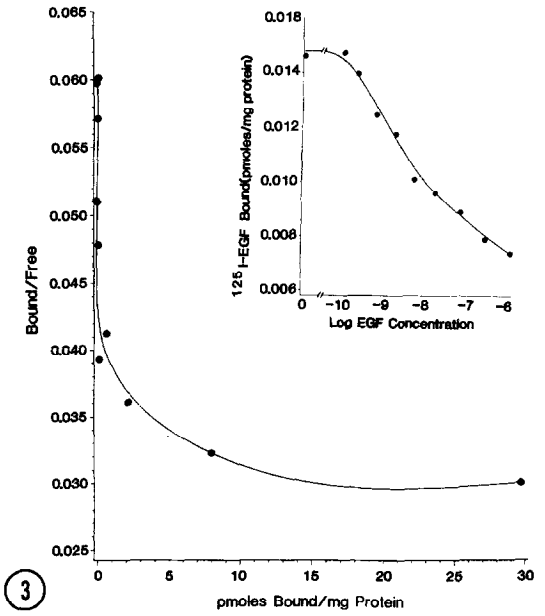
Fig. 2 **Time course of ^{125}I-EGF binding to cortical basolateral membranes** (left panel) **and to cortical luminal membranes** (right panel). 100 µg of membrane protein were incubated at 22° C for 120 minutes, at pH 7.5. ^{125}I-EGF binding was assessed in the presence of 2.5 x 10^{-10} M EGF, and in the presence and absence of 1 x 10^{-6} M unlabeled EGF. Specific binding (shown as triangles) is expressed as the difference between total (shown as squares) and non-specific binding (shown as circles).

basolateral membranes. The specific binding was about 80% of the total binding and reached saturation at 30 minutes incubation. The specific binding to cortical basolateral membranes was pH dependent (maximum, pH 7.5), temperature dependent (maximum at 22°C) and was linear with protein concentration in the range of 50-350 µg protein (results are not shown). Figure 2 (right panel) shows the binding of ^{125}I-EGF to cortical luminal membranes. In contrast to cortical basolateral membranes, there was only 30% specific binding of ^{125}I-EGF to cortical luminal membranes and this amount of binding can be attributed to cross-contamination of the luminal with basolateral membranes (as determined by formula for cross contamination in the Methods).

Figure 3 shows the Scatchard analysis of a representative experiment of ^{125}I-EGF binding to cortical basolateral membranes. The insert shows the displacement curve from which the Scatchard analysis was derived. Two distinct classes of binding sites in cortical basolateral membranes are apparent: a high affinity and low affinity binding sites. Similar results were seen in 8 other experiments and Scatchard analysis of all the experiments revealed high affinity binding sites with Kd of 29 ± 7 nM and Bmax of 0.49 ± 0.09 pmol/mg protein; and low affinity binding sites with Kd of 5.0 ± 1.1 nM and Bmax of 86.5 ± 25.3 pmol/mg protein. Thus, these studies expand the observations of EGF binding in

proximal tubule cell cultures (3) by localizing this binding to basolateral but not to luminal cortical membranes.

To further characterize EGF binding to renal plasma membranes we studied ^{125}I-EGF binding to medullary plasma membranes. As shown in Figure 4 the specific binding to medullary plasma membranes was 85% of total binding, a higher value than that observed in the cortical basolateral membranes. The high specific binding to medullary plasma membranes is of great interest because preproEGF has been localized to the thick ascending limb of Henle (2) and our medullary plasma membranes likely contain this structure. These results are also in agreement with other studies utilizing immunoelectron microscopy which have demonstrated EGF immunoreactivity on the apical membrane of the thick ascending limb of Henle (9). Since our medullary plasma membranes are a mixture of luminal and basolateral membranes it is unclear whether the medullary binding sites are located in either one of the membranes (luminal or basolateral) or in both membranes.



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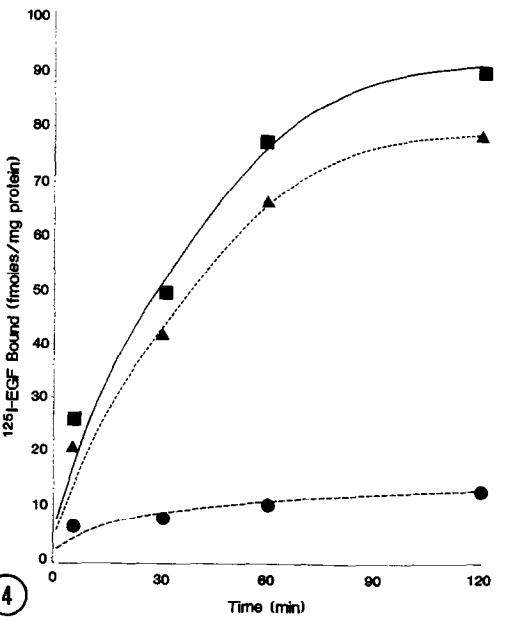

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Fig. 3 **Scatchard analysis of ^{125}I-EGF binding to cortical basolateral membranes:** Experiments were performed by varying the concentration of unlabeled EGF from 0 to 1 x 10^{-6} M. Kinetics of binding were determined by linear regression analysis of high and low affinity binding sites. The displacement curve from which the Scatchard analysis was derived is shown in the insert.

Fig. 4 **Time course of ^{125}I-EGF binding to medullary plasma membranes:** Details are given in the legend for Figure 2.

In summary, we have shown for the first time the presence of specific high affinity binding sites for EGF in purified renal cortical basolateral, but not in cortical luminal membranes. In addition, we have shown the presence of specific binding sites for EGF in medullary plasma membranes. These results are consistent with a physiologic role of EGF in proximal tubule and in medullary portions of the nephron which requires further investigation.

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