The Renal Handling of Biologically Active Peptides

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The site of protein elimination and degradation is until now not exactly known. The reticulo-endothelial system may play a major role (Ehrenreich and Cohn, 1967). Emphasis has been put on the kidney as an organ for the elimination and catabolism of proteins, but data on plasma disappearance after bilateral nephrectomy of, for example, albumin, IgG or transferrin (Seward and Morgan, 1970; Rosenfeld et al., 1962) show clearly that the kidney plays no more than a minor role in the elimination of these proteins. Studies on the arteriovenous balance of -amino-nitrogen across the kidney failed to reflect significant proteolysis (Elisch et al., 1955).

However several exceptions from this statement must be made:
(1) Overload of the kidney with a protein, due to glomerular diseases leads to an accumulation within proximal tubular cells and subsequent degradation.
(2) Exogenous proteins such as Staphylococcal Enterotoxin B (Norman and Stone, 1972) or endogenous pathologic proteins such as the Bence Jones protein (Wochner et al., 1967) are rapidly taken up by the kidney and degraded, provided their molecular weight allows the passage through the glomerular filter. Experiments with intraluminal injections of various foreign proteins - horseradish peroxidase, ferritin - demonstrated the absorptive capability of the proximal tubule cells for proteins that usually do not appear in the primary urine.
(3) The kidney is the major site of elimination and degradation for the peptide hormones: insulin and glucagon (Nhr et al., 1958); human chorionic gonadotrophin (Collip et al., 1966); growth hormone and luteinizing hormone (De Kretser et al., 1969); luteinizing hormone - releasing hormone (Miychi et al., 1973), and prolactin (Rjniemi et al., 1974) have been shown to accumulate in the kidney cortex. All these hormones have very short half-lives of several minutes as they are rapidly catabolized in the kidney. This process warrants the rapid removal rates necessary for components of biological feedback mechanisms.

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Peptides and proteins thus enriched in the kidney, have been found nearly exclusively in the proximal tubule cell. It is generally accepted that the mechanism by which the peptides enter the tubule cell is pinocytosis, which occurs mainly in the proximal tubules. The distal tubule may participate in the reab•
sorptive process only during a very high overload with the protein (Klockrs et al., 1974). The sequence of events following ingestion of a protein by the tubule cell has mostly been studied by morphological techniques using histochemistry, electron microscopy and autoradiography (Ericsson, 1965; Münsch, 1966; Strus, 1967; Thoenes et al., 1970). Biochemical experiments with isolated subcellular fractions demonstrated the significant role of lysosomes in the degradation of ingested material (Normn nd Stone, 1972). From these experiments it is also known that denaturation of a protein increases its catabolism (Cnonico, 1973), but little is known on the biochemical level about the dynamic processes that precede catabolism.

We found that the protease inhibitor from bovine organs - a basic peptide with a molecular weight of 6,500 which is sold under the trade name Trasylol® - may serve as a useful tool to study the renal handling of peptides in general (Hbermn, 1973). The affinity of the kidney for the protease inhibitor is very high. Up to 5 mg of the peptide may be injected intravenously into rats without producing overload peptiduria. The protease inhibitor has been localized by immunofluorescence technique (Török, 1972) at the proximal tubular brush-border membrane and in close relation to periodic acid Schiff positive granules which move from the subapical towards the basal region of the cells. However, with this method, only a suggestive evidence can be obtained on the pinocytic transport of the peptide. Therefore we tried to further characterize these results by biochemical experiments.

In a study on binding of the protease inhibitor to an isolated rat-kidney brush-border fraction (Just nd Hbermn, 1973) we gave biochemical data for the exceptional capacity and affinity of the brush border for the inhibitor. Binding was saturable, the binding capacity being 120 µg (= 18 nmol) of peptide per mg of membrane protein. Treatment of the brush border with sialidase markedly reduced binding. These findings are in agreement with the results of Stoddrt nd Kiernn (1973), who found that the protease inhibitor binds strongly to glycoproteins containing sialosyl groups. This interaction appears to be the initial step of the reabsorption of the peptide.

In order to get quantitative biochemical data on the subsequent process, we injected rats with the ¹² I-labeled protease inhibitor. At different times thereafter we fractionated the kidneys by differential and sucrose gradient centrifugation. The fractions were identified by marker enzymes, density and radioactivity. We expected the injected peptide once adsorbed or incorporated by a subcellular structure in vivo to be recovered together with the structure by in vitro isolation. Our purpose was to follow the change in the location of the protease inhibitor with time, within the sucrose gradient.
The first picture shows the distribution of marker enzymes in the gradient. This continuous gradient from 1.1 to 1.9 molar sucrose has been used by Maunsbach for the isolation of rat kidney lysosomes. Maximum activities of the various enzymes appear at the following densities: endoplasmic reticulum at 1:14; brush-border membrane at 1:16; mitochondria at 1:17-1:18; lysosomes at 1:191:20.

The lysosomal enzyme -glucuronidase appears in a second peak in the soluble fraction, which is in consistence with the known lysis of lysosomes during protein digestion.

The distribution of the $^{12}$I-protease inhibitor within the gradient strongly depends on the time of sacrifice after the injection of the peptide. In general, with increasing survival periods, the radioactivity moves from a region of lower density towards higher densities.

2.5 min after injection, the radioactivity follows the distribution pattern of the brush-border enzymes. With 1 min, radioactivity and enzyme activity run even more parallel. 20 min after injection the distribution of radioactivity shows two peaks. The major portion reaches fractions of the higher density which contain the lysosomal enzyme activity. The other portion follows the brush-border enzyme as before. After 30 min the picture is very similar, the peaks being equally high. 24 h after injection the portion of the higher density has nearly completely disappeared.

The two following pictures again show the time-dependent distribution of radioactivity (fig. 1, 2). 1 min after injection radioactivity is recovered only with the brush-border membrane (fig. 1). After 5 min, radioactivity has additionally moved into higher densities via a broad shoulder. With 20 and 30 min one brush border and one lysosomal peak appear. 3 h after injection the lysosomal portion decreases and within 24 h it has disappeared (fig. 2).

In order to examine any possible phenomena of redistribution of the peptide, we added the $^{12}$I-protease inhibitor in vitro to the kidney homogenate. The results showed the parallel distribution of the peptide with the brush border as was expected from our binding data.

The particle-bound radioactivity was subsequently extracted from the fractions and measured for their immunological reactivity. According to these results, the radioactivity in the gradient represents immunologically intact protease inhibitor. In order to find out if other peptides show a comparable behavior, we studied the distribution of $^{12}$I-labeled insulin with the same technique (fig. 3).

0.5 min after injection of 8-ng insulin the major portion of the radioactivity follows the brush-border enzymes, additionally an appreciable amount exists in the soluble fraction. 5 min after injection the radioactivity is broadly distributed throughout the gradient. With 10 and 25 min a significant lysosomal peak ap-
Fig. 1, 2. Sucrose density gradient fractionation of a crude rat kidney cortical fraction at various times after i.v. administration of 0.1 mg $^{12}$I-protease inhibitor. Continuous sucrose density gradient (1.1-1.9 M) from right to left.

Fig. 3. Sucrose density gradient fractionation of a crude rat kidney cortical fraction at various times after i.v. administration of 8 ng $^{12}$I-insulin.

pears. In the control experiment, the major portion of the peptide is unbound in the soluble fraction, whereas a minor portion binds to the brush border. This result is in agreement with our binding studies, where insulin is bound to the brush-border membrane to a lesser extent and by a non-saturable manner, as compared with the protease inhibitor.

We studied further the nature of the particles of higher density which were associated with the peptide after longer survival periods. According to Munsbch (1966 b), a primary granule fraction from rat kidneys was isolated following intravenous injection of either horseradish peroxidase, insulin or protease inhibitor, and purified by the same sucrose gradient as used before. The specific activity of the lysosomal enzyme acid phosphatase showed a very similar distribution pattern to the specific radioactivity. This is the case for horseradish peroxidase, insulin and protease inhibitor as well. Thus, the transport of peptides in the kidney can be described on the biochemical level in the following three steps:

1. Binding to the brush border as the initial step of reabsorption.
2. Incorporation in micropinocytic vesicles lacking any enzyme activity.
3. Transport in phagolysosomes.

The basic protease inhibitor exhibits several advantages for the study of the renal handling of peptides:

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1. The chemical and biological stability of the $^{12}$I-labeled peptide is very high.
2. The kidney has an exceptional high affinity for the peptide.
3. In contrast to endogenous peptide hormones, the protease inhibitor is catabolized very slowly in the kidney. There is still some immunoreactive material present, 6 days after injection.

Summary
With the use of the protease inhibitor from bovine organs Trasylol® -- as a model, we studied the pinocytic transport of peptides in the kidney. Rats were injected with the $^{12}$ I-labeled peptide and killed at different times thereafter. Kidney homogenates were subfractionated by differential and sucrose gradient centrifugation. Radioactivity was measured in the fractions in order to study the time-dependent fixation of the peptide to different cell organelles. With short survival periods, the protease inhibitor is recovered in the brush-border fraction, with longer periods, a shift towards the lysosome fraction takes place. Thus, the renal transport of the protease inhibitor consists of three steps: binding to the brush border, incorporation in micropinoocytic vesicles and transport in phagolysosomes.

References


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