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Effects of Proteolytic Enzymes on Uremic Serum Inhibition of Hepatic and Renal Transport Functions (39016)

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We have previously reported that rat uremic serum or dialysates of uremic serum can inhibit the: (a) uptake of paraaminohippurate by rat renal cortical slices (1), and (b) uptake of purines (xanthine, hypoxanthine) by rat liver slices (2).

The present studies were prompted by several questions: (a) Are these inhibitory effects on renal and hepatic transport functions caused by one substance or several? (b) Might any of these effects be the result of a substance containing peptide bonds? (c) Might there be a differential proteolytic destruction of the inhibitory effects on renal and hepatic transport functions, thus strengthening the likelihood that more than one inhibitory substance is involved?

In an attempt to answer these questions, dialysates of rat uremic serum were incubated with each of four proteolytic enzymes: Pronase (derived from Streptomyces griseus), carboxypeptidase A, L-aminopeptidase, or trypsin.

We present data showing that of the enzymes studied, Pronase produced the greatest destruction (63.2 ± 4.5%) of the inhibitory activity of uremic dialysates on renal transport, with a much smaller destruction (17.8 ± 2.1%) of the inhibitory effect on hepatic transport.

Materials and methods. The renal uptake of para-aminohippurate was measured in terms of S/M (slice:medium) ratios (1), while hepatic uptake of purines was measured in terms of "augmented allantoin production" (2).

The uremic serum (average BUN = 295.0 mg%) used in these studies was obtained from male CFN Carworth albino rats 48 hr after they had undergone bilateral nephrectomy (2). Normal serum was obtained from control animals 48 hr after they underwent sham operations. As in previous studies (1, 2) food was withheld during the 48-hr period following surgery on experimental and control animals while water was allowed *ad libitum*.

In the studies of renal transport functions, dialysates of pooled normal or uremic serum were prepared by dialysing the serum against Cross-Taggart solution for 24 hr. One ml of dialysate equaled 1.0 ml of serum in all studies of renal or hepatic transport. Dialysates of pooled normal or uremic serum for the experiments dealing with hepatic transport were prepared with Krebs–Ringer–phosphate solution, as reported previously (2). One ml of dialysate was added to a final volume of 2.7 ml in the renal and 3.0 ml in the hepatic studies.

Cross–Taggart solution served as the ambient medium in studies of renal uptake of para-aminohippurate while Krebs–Ringer–phosphate solution was used in the studies of hepatic transport.

Uremic inhibition was measured in every instance by paired studies employing dialysates of normal and uremic sera. In two series of experiments, each consisting of 22 observations, 1 ml of dialysate of uremic serum produced a 68.8 ± 0.5% inhibition of renal uptake of para-aminohippurate and a 62.6 ± 0.8% inhibition of hepatic uptake of purines.

Four proteolytic enzymes were studied with procedures described in the cited references: Pronase (Calbiochem) (3), Carboxypeptidase A (Calbiochem) (4), Leucine Aminopeptidase (Sigma) (5), and Trypsin (Calbiochem) (3). Dialysates of normal or uremic serum were incubated with each of these four enzymes for 96 hr. The incubation temperatures were 25° with carboxypeptidase

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TABLE I. EFFECT OF ENZYMATIC TREATMENT OF SERUM DIALYSATES ON UREMIC INHIBITION OF RENAL PARAAMINOHIPPURATE (PAH) UPTAKE[a].

Enzyme	Renal uptake of PAH (slice:medium ratios)				Destruction[b] (%)
	Nonenzyme treated		Enzyme treated		
	Normal (N)	Uremic (U)	Normal (N)	Uremic (U)	
Pronase	8.5 ± .13	2.6 ± .09	8.1 ± .19	6.0 ± .15	63.2 ± 4.5[c]
Carboxypeptidase-A	8.4 ± .11	2.6 ± .07	8.3 ± .10	4.0 ± .23	25.2 ± 3.8[c]
L-aminopeptidase	8.6 ± .12	2.7 ± .08	8.4 ± .17	3.4 ± .08	12.5 ± 1.5[c]
Trypsin	8.9 ± .06	2.9 ± .16	8.6 ± .13	3.2 ± .10	5.8 ± 1.5[c]

[a] Values are means ± standard error of mean. Each value represents the average of six experiments.
[b] Destruction (%) = (% inhibition of untreated dialysate − (% inhibition of treated dialysate)/(% inhibition of untreated dialysate), where % inhibition = 100 × (N-U/N) ("paired differences").
[c] Statistically significant (method of "paired differences").

TABLE II. EFFECT OF ENZYMATIC TREATMENT OF SERUM DIALYSATES ON UREMIC INHIBITION OF HEPATIC ALLANTOIN PRODUCTION[a].

Enzyme	Augmented allantoin production (mg/100 mg)				Destruction[b] (%)
	Nonenzyme treated		Enzyme treated		
	Normal (N)	Uremic (U)	Normal (N)	Uremic (U)	
Pronase	123.5 ± 2.6	45.2 ± 1.2	119.0 ± 2.3	57.0 ± 1.7	17.8 ± 2.1[c]
Carboxypeptidase-A	127.0 ± 2.0	49.5 ± 1.6	125.0 ± 2.1	53.3 ± 1.0	5.7 ± 2.2[c]
L-aminopeptidase	131.6 ± 1.2	51.2 ± 2.5	130.6 ± 1.7	57.6 ± 2.2	8.4 ± 1.4[d]
Trypsin	127.4 ± 1.8	44.6 ± 2.9	122.2 ± 1.1	46.6 ± 2.3	4.9 ± 2.1[d]

[a] Values are means ± standard error of mean. For Pronase and Carboxypeptidase A, each mean represents the average of six experiments, and for L-Aminopeptidase and Trypsin, 5.
[b] Destruction (%) = (% inhibition of untreated dialysate) − (% inhibition of treated dialysate)/(% inhibition of untreated dialysate), where % inhibition = 100 × N-U/N ("paired differences").
[c] Statistically significant, by method of "paired differences."
[d] Not statistically significant, by method of "paired differences."

and 37° for the other three enzymes. A pH of 8.1 was employed with leucine aminopeptidase, and corrected to pH 7.4 before tissue slices were incubated with the enzyme-treated dialysates. With the other 3 enzymes, the 96-hr incubation was carried out at pH 7.4.

The effects of the proteolytic enzymes on the dialysates were studied in sets of four simultaneous measurements made with hepatic or renal tissue obtained from one animal. Each set employed enzyme-treated and nontreated dialysates of normal and uremic serum.

Percentage destruction of inhibitory activity of uremic dialysates by enzymes was calculated as shown in the footnotes to Tables I and II.

Results. Table I shows the extent to which the four enzymes destroyed the inhibitory actions of dialysates on the renal uptake of para-aminohippurate (PAH). Pronase exerted the maximal reduction of uremic inhibition, 63.2 ± 4.5%.

Table II demonstrates the ability of the 4 enzymes to destroy the uremic inhibition of hepatic uptake of purines, as measured by augmented hepatic allantoin production. Here, too, Pronase exerted the maximal destructive effect, 17.8 ± 2.1%. This effect is

much less than that on the renal inhibition of PAH uptake, 63.2 ± 4.5%.

Differences between the enzymatic destruction of uremic dialysate inhibition of renal uptake of PAH and of hepatic allantoin production were analyzed statistically with the use of the "unpaired difference" method. Statistical significance was demonstrated for the difference in the destructive effects of Pronase ($P < .001$) or Carboxypeptidase A ($.001 < P < .005$) while the differences with Leucine Aminopeptidase ($.2 < P < .4$) or Trypsin ($P > 0.5$) were not statistically significant.

Discussion. The data presented in this paper demonstrate that dialysates of uremic serum, under the experimental conditions employed, are approximately equal in their inhibitory effects on the renal uptake of paraaminohippurate (68.8 ± 0.5%) and on the hepatic uptake of purines (62.6 ± 0.8%).

Therefore, this equality of inhibitory effects makes it possible to compare quantitatively the ability of the various proteolytic enzymes investigated to destroy the inhibitory effects of dialysates of uremic serum on the aforementioned renal and hepatic transport functions.

As stated, Pronase produced the greatest destruction (63.2 ± 4.5%) of the inhibitory action of uremic serum dialysates on renal PAH transport, with a much smaller destruction (17.8 ± 2.1%) of the inhibitory effect on hepatic transport. This suggests that the inhibitory activity of dialysates of uremic serum on the renal transport of paraaminohippurate is largely related to a peptide structure.

On the basis of this observed differential enzymatic destruction, two broad possibilities exist concerning the number of compounds responsible for the inhibitory activity of uremic serum on hepatic and renal transport functions: (a) At least two exist, one acting as an inhibitor of renal transport and the other as an inhibitor of hepatic transport; or (b) one compound exists, and when it is split by Pronase, one fragment is still largely capable of inhibiting hepatic transport, but not renal transport.

Tables I and II show that the effects of dialysates of normal serum on renal and hepatic transport functions are the same for enzyme treated and untreated samples of dialysates. This fact excludes the possibility that the proteolytic enzymes had any direct effects on the tissue slices themselves.

Summary. Dialysates of rat uremic serum were incubated with Pronase, Carboxypeptidase A, Leucine Aminopeptidase, or Trypsin to investigate whether these enzymes might destroy the inhibitory effects of the dialysates on the uptake of paraaminohippurate by rat renal cortical slices or the uptake of purines by rat liver slices. Of these enzymes, Pronase produced the greatest destruction (63.2 ± 4.5%) of the inhibitory action on renal transport, with a much smaller destruction (17.8 ± 2.1%) of the inhibitory effect on hepatic transport.

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