Variations in the Activity of Renal and Hepatic Cysteine Cathepsins

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Activities of the cysteine cathepsins B, L, and especially H in hepatic and renal tissues from pregnant rats were found to be many times higher than in those from nonpregnant controls. In rats fasted for several days, these activities declined in the kidneys and rose in the liver.

Key Words: *proteolysis; cathepsins; pregnancy; fasting*

In the past two decades, many new insights have been gained into the functional capabilities of lysosomal proteolysis. In particular, the lysosomal cysteine cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), and L (EC 3.4.22.15) have been shown to participate not only in total destructive proteolysis but also in the limited proteolysis taking place when the precursors of active molecules are processed [3-7].

Of considerable interest is the contribution of cathepsins to cellular proteolysis under functional loads and in disease states involving protein deficiency or, on the contrary, protein overload of cells. In rats deprived of food for only 24 h, cathepsin B activity in the liver was found to be tens of times higher than in fed rats [2]. We detected greatly elevated cathepsin B activities in homogenates of biopsy specimens from patients with progressive proteinuria and showed that the rise in the activity of this enzyme can be restrained by endogenous proteolysis inhibitors of various origin. The study described here was designed to determine the relative contributions of different thiol-dependent cathepsins to proteolytic functions of the liver and kidneys in rats deficient in plastic material due to pregnancy and/or starvation.

MATERIALS AND METHODS

The activities of cysteine cathepsins were measured as previously described [1] with respect to the following three substrates: Na-benzoyl-DL-arginine p-nitroanilide (BAPNA), DL-leucine p-nitroanilide (LEUPA) (both from *Khimreaktiv*, Moscow), and a chromogenic casein preparation (designated OKA-AS-K) (Vilnius). In the first part of the study, the specific activities of cathepsins B, H, and L isolated from human, canine, porcine, and bovine kidneys by a procedure we had developed earlier [1] were measured after thorough purification. In the second part, activities of these cysteine cathepsins were estimated in homogenates prepared from renal and hepatic tissues of random-bred female white rats. Five groups of rats were used. The first (control) group consisted of females in different periods of the reproductive cycle maintained on a standard diet. Rats of groups 2 and 3 received only water during 3 and 7 days, respectively, before sacrifice. Group 4 consisted of normally fed rats pregnant for >7 days before sacrifice while group 5 comprised rats pregnant for >7 days and fasted for 3 days before sacrifice (in these two groups, embryos were examined at autopsy to evaluate organogenesis).

Prior to the preparation of homogenates, all livers and kidneys removed from the rats were stored in a freezer at -18°C. Homogenates were prepared in a glass homogenizer using a 0.01 M sodium phosphate buffer, pH 6.4, containing 5 mM EDTA (sodium salt) and 0.1% butanol by volume. The ratio of hepatic and renal tissues to the buffer was 10 mg tissue:1 ml buffer. The homogenizer was cooled with running water. The homogenates were kept at 4°C for 1 h after preparation, including a 20-min centrifugation at 6000 rpm. Cathepsin activities were estimated in the supernatants,

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as were protein concentrations (by Lowry's method). The results were subjected to conventional statistical treatment using a computer.

RESULTS

The purification procedure we used [1] yielded highly purified preparations of cathepsins B, H, and L from human, porcine, bovine, and canine renal tissues. As can be seen in Table 1, the major fractions of each of the three cysteine cathepsins exhibited more or less equal activities toward the same substrate (activities of their minor fractions are not shown here). This indicates that these peptide hydrolases from different sources were indeed highly purified and have similar properties, and suggests that the proposed substrates can be used for estimating the activities of cysteine cathepsins in their various combinations in unpurified material. According to the data in Table 1, none of the major cathepsin B or L fractions were active with respect to LEUPA. The reported ability of renal cathepsin B to display appreciable aminopeptidase activity [7] can be attributed to insufficient separation of cathepsins in the course of their purification. It follows from Table 1 that the use of LEUPA as substrate should enable the specific activity of cathepsin H to be demonstrated in most instances of analysis using homogenates of animal tissues.

Thiol-dependent BAPNA hydrolase activity has been reported by some authors to bind not only to cathepsin B but also to cathepsin H [7]. Judging by the data in Table 1, the major contribution to the thiol-dependent hydrolysis of BAPNA is most likely made by cathepsin B, while the contribution of cathepsin H may be disregarded in considering this activity. Casein substrates are customarily employed for detecting the activity of cathepsin L [2]. Since the purified preparations of cathepsin B and, to a lesser extent, those of cathepsin H showed high affinity for OKA-AS-K under the same conditions as did cathepsin L, the latter activity of organ homogenates should be interpreted taking into account the BAPNA and LEUPA hydrolase activities. Thus, the concomitant use of these three substrates yielded a clearer picture of the spectrum of cysteine cathepsins in mammalian organ homogenates.

In the second part of the study we were able to record maximal activities of the cathepsins in hepatic and renal tissue homogenates owing to the presence of cysteine in a concentration of 4 mM. The results are summarized in Table 2. In the nonpregnant fasted rats (groups 2 and 3), variations of cathepsin activities in the liver were opposite to those in the kidneys. Thus, the 7-day fast led to significant rises in the liver and significant falls in the kidneys, the greatest fall (by 30%) being recorded for cathepsin L activity toward OKA-AS-K. Although the greatest change occurred in the activity toward this substrate, the overall (absolute) change in thiol-dependent activity was recorded for the activity toward LEUPA. Cathepsin activities vis-a-vis all three substrates varied in the same directions.

The finding that the changes in cathepsin activities in the liver were opposite to those in the kidneys during fasting points to different roles of these two organs in endogenous nutrition when plastic material is in short supply. Although the levels of cysteine cathepsin activity in the kidneys are initially higher than in the liver, the latter begins to play a more important role in sustaining the amino acid pool upon the transfer to endogenous nutrition at the expense of internal reserves. Activation of renal thiol-dependent proteolysis was found upon analysis of biopsy specimens from patients with the nephrotic syndrome [3]. The depression of cysteine cathepsin activities in renal tissues from fasted rats is relatively slight when their values are compared to those in the control group.

TABLE 1. Specific Activities of Cysteine Cathepsins (Major Fractions) Isolated from Human and Animal Kidneys and Purified Using a Multistage Procedure

Cathepsin		Specific activity with respect to substrates:		
		BAPNA (pH 6.4), μmol/min×mg protein	LEUPA (pH 6.4), μmol/min×mg protein	OKA-AS-K (pH 5.1), μg/min×mg protein
H:	human	$9.0\pm1.0\times10^{-3}$	0.601±0.008	2.80±0.08
	porcine	$8.0\pm1.0\times10^{-3}$	0.580±0.010	2.60±0.06
	bovine	$6.0\pm2.0\times10^{-3}$	0.610±0.015	2.42±0.14
	canine	$11.0\pm2.0\times10^{-3}$	0.640±0.015	3.22±0.12
B:	human	0.350±0.020	Undetectable	12.60±0.10
	porcine	0.345±0.010	Undetectable	12.02±0.11
	bovine	0.305±0.025	Undetectable	8.07±0.18
	canine	0.375±0.015	Undetectable	14.12±0.21
L:	human	2×10^{-3}	Undetectable	13.60±0.14
	porcine	1×10^{-3}	Undetectable	13.22±0.14
	bovine	1×10^{-3}	Undetectable	Not determined
	canine	3×10^{-3}	Undetectable	14.06±0.18

TABLE 2. Activities of Thiol–Dependent Proteinases in Homogenates of Livers and Kidneys from Pregnant and/or Fasted Rats

Group (*n*)	Thiol-dependent activity with respect to					
	BAPNA, nmol/min×mg protein		LEUPA, nmol/min×mg protein		OKA-AS-K, μg/min×mg protein	
	liver	kidneys	liver	kidneys	liver	kidneys
Control (12)	$0.082\pm3.84\cdot10^{-3}$	$0.380\pm4.63\cdot10^{-3}$	$6.16\pm1.32\cdot10^{-3}$	14.73±0.83	$1.93\pm1.3\cdot10^{-1}$	$3.60\pm9.0\cdot10^{-2}$
Fasted for 3 days (12)	$0.096\pm4.97\cdot10^{-3**}$	$0.321\pm4.57\cdot10^{-3***}$	$8.83\pm4.43\cdot10^{-1****}$	16.52±0.09**	$2.20\pm5.54\cdot10^{-1****}$	$3.52\pm4.82\cdot10^{-2}$
Fasted for 7 days (12)	$0.128\pm5.14\cdot10^{-3***}$	$0.262\pm5.47\cdot10^{-3***}$	$9.57\pm3.00\cdot10^{-1****}$	11.08±0.2***	$2.99\pm1.25\cdot10^{-1****}$	$2.20\pm6.33\cdot10^{-2***}$
Pregnant for >7 days (6)	$0.843\pm2.40\cdot10^{-2****}$	$0.637\pm1.53\cdot10^{-2****}$	168.3±4.09****	91.13±1.66****	$17.55\pm9.6\cdot10^{-1****}$	$9.41\pm4.6\cdot10.0^{-1****}$
Pregnant for >7 days and fasted for 3 days (7)	$0.449\pm3.34\cdot10^{-2****}$	$0.551\pm1.15\cdot10^{-2****}$	81.04±3.27****	64.10±0.84****	$7.89\pm3.07\cdot10^{-1****}$	$5.73\pm9.75\cdot10^{-2****}$

Note. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ in comparison with the control group; the $^{+}$ and $^{-}$ signs denote increases and decreases in the values.

Much greater alterations in cathepsin activities occurred in pregnant rats, particularly after the 3-day fast (Table 2). Indeed, these activities in the group of fasted pregnant rats were many times higher than in the control group, in both liver and kidneys. Stimuli regulating the reduction of renal proteolysis in pregnant animals during fasting are far from being sufficient for lowering cathepsin activities to levels approximating those in controls. During pregnancy and fasting, variations of these activities were more marked in the liver than in the kidneys. The data obtained for individual enzymes showed a greater activation of cathepsin H than of the other two enzymes. The observation that fasting reduces hepatic cathepsin activities in pregnant rats and raises them in nonpregnant animals highlights the differential roles of the liver and kidneys in the maintenance of endogenous proteolysis under abnormal conditions. Apparently, placental factors activating maternal cellular proteolysis are secreted at the stage of fetal organogenesis, their action possibly being somewhat limited because the reserve forms of plastic material are depleted (e.g., during fasting).

To summarize, regulatory mechanisms appear to exist through which cysteine cathepsins can increase their activity many times in the liver and kidneys. During pregnancy the activation of cathepsin H in these organs far exceeds that of cathepsins B and L. The regulatory stimuli geared toward reducing cathepsin activities act on the liver in a manner distinct from their action on the kidneys. Finally, the activities of different cathepsins vary unequally, the greatest variations being undergone by cathepsin H, in both liver and kidneys.

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REFERENCES

1. A. A. Zhloba, *Ukr. Biokhim. Zh.*, **63**, № 2, 45-51 (1991).
2. F. M. Baccino, L. Tessitore, and G. Cecchini, *Biochem. J.*, **206**, № 2, 395-405 (1982).
3. P. Bohley, S. Riemann, H. Kirschke, *et al.*, in: *Cell Differentiation in Microorganisms, Plants and Animals*, Amsterdam (1977), pp. 225-242.
4. H. Kirschke, J. Langner, B. Wiederanders, *et al.*, *Eur. J. Biochem.*, **74**, № 2, 293-301 (1977).
5. C. Lazure, N. G. Seidan, D. Pelaprat, and M. Chretien, *Can. J. Biochem. Cell Biol.*, **67**, № 7, 501-515 (1983).
6. P. S. Quinn and J. D. Judah, *Biochem. J.*, **172**, 309 (1978).
7. G. J. Thomas, K. T. Hughes, and M. Davies, *Biochem. Soc. Trans.*, **8**, 596-597 (1980).