Corticosteroid Effect on Hepatic Collagens

Following the administration of a suitable doses of corticosteroids, hepatic content of collagen is reduced, especially under pathological conditions[1–5]. The action of corticosteroids may be analyzed from the aspect of both fibroblasts and liver cells, yet the exact mechanism for a loss of hepatic collagens remains obscure. The present report undertook to elucidate the effect of corticosteroid on hepatic collagens.

Male Wistar strain rats with an average weight of 200 g, maintained on Clea MF rat diet were divided into 2 groups. The control group received physiological saline i.m. daily for 5 days. The cortisone group received 1 mg of cortisone acetate per 100 g body weight i.m. daily for 5 days. The animals were sacrificed 1 day after the last treatment. Determination of hepatic contents of collagens and their specific radioactivities are described elsewhere[6]. Briefly, 1 g of liver slices was incubated in vitro with 10 μC of L-^{14}C-proline for 60 min. The labelled liver was homogenized and fractionated into 3 collagen fractions by the method of JACKSON[7]. Each collagen fraction was extracted with 5% trichloroacetic acid at 90 °C for 30 min[8]. From the hydrolyzed collagen fractions, ^{14}C-hydroyxproline was isolated by the method of PETERKOFSKY and PROCKOP[9], and hydroxyproline content was determined by the method of STEGEMANN and STALBER[10].

The collagenolytic activities towards soluble and insoluble collagens, which differ from collagenase, were assayed as previously described[6,11]. Namely the collagen fractions used as substrates were obtained from rat skin labelled with L-^{14}C-proline in vivo. The specific radioactivities were 1560 for acid soluble collagen and 1993 for insoluble collagen in a unit of dpm/mg of protein respectively. The substrates were mixed with liver homogenates in 0.1 *M* sodium acetate buffer, pH 4.0, followed by incubation for 18 h at 35 °C. The collagenolytic activity towards acid soluble collagen was assayed from the radioactivity of the ethanol soluble fraction, while the activity towards insoluble collagen was determined from the radioactivity of the supernatant obtained by the centrifugation at 120,000 × *g* for 30 min. To assess the intracellular distribution of the collagenolytic activity, rat liver homogenates were fractionated into nuclear, mitochondrial lysosomal, microsomal and supernatant fraction by the method of DE DUVE et al.[12]. Protein content and the collagenolytic activity towards acid soluble collagen were determined on each subcellular fraction. Before enzyme assay each fraction was treated with Triton × 100 (final concentration 0.1%).

The results are shown in the Table. Cortisone acetate administration caused a reduction of contents and specific radioactivities of hepatic collagens especially neutral soluble collagen. The results coincide approximately with the findings concerning rat skin collagens in the same condition[13–15], probably through the interference with mRNA activity or the inhibition of ribosomal aggregation in fibroblasts[16]. On the other hand, cortisone group showed an elevated collagenolytic activity towards acid soluble collagen but showed no change in the activity of solubilizing insoluble collagen. As shown in the Figure, the induced collagenolytic activity was mainly recovered in nuclear and lysosomal fraction. The results indicate that cortisone administration induces nuclear and lysosomal collagenolytic activity.

Two enzyme systems capable of collagen degradation were found in the liver: a lysosomal collagenolytic activity, operative at acid pH[6,11] and a collagenase activity, operative at neutral pH[17]. The latter, which is measured

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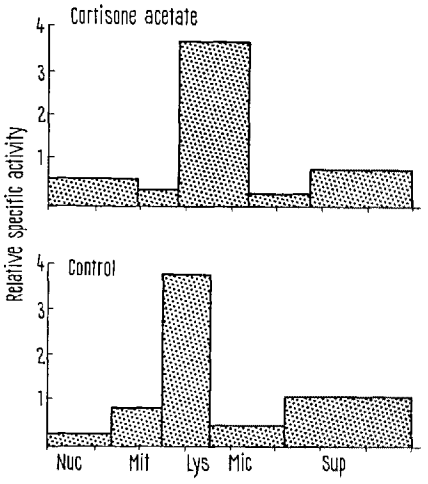
Cortisone effect on hepatic collagens

	Controls		Cortisone acetate
Contents of collagen (μg hydroxyproline/g liver)	(5)		(5)
Collagen	142 ± 19		126 ± 17
Neutral soluble collagen	5.0 ± 0.9	($p < 0.01$)	1.7 ± 0.2
Acid soluble collagen	7.1 ± 3.7		3.8 ± 0.7
Insoluble collagen	132 ± 22		116 ± 32
Recovery (%)	105 ± 10		93 ± 14
$^{-3}$10 × specific radioactivities of collagen (dpm/μmole hydroxyproline)	(5)		(5)
Collagen	8.8 ± 1.5	($p < 0.01$)	4.4 ± 0.7
Neutral soluble collagen	233 ± 96	($p < 0.05$)	112 ± 15
Acid soluble collagen	69 ± 38		45 ± 18
Insoluble collagen	0.83 ± 0.16		1.62 ± 0.93
$^{-3}$10 × collagenolytic activities (dpm/g liver)			
Acid soluble collagen	2.09 ± 0.85 (8)	($p < 0.01$)	3.80 ± 0.83 (5)
Insoluble collagen	27.6 ± 3.3 (5)		28.4 ± 2.8 (5)

Figures in parenthesis denote number of animals. Results are given as means ± S.D.

to be very weak in the liver, seems to arise from mesenchymal granulocytes[18]. It was reported that corticosteroid was able to induce extracellular collagenolytic activity in skin or fibroblasts[14,19]. Present results indicate also that cortisone administration caused an increase of the lysosomal collagenolytic activity in the liver, yet it remains obscure whether the activity originates from hepatocytes or mesenchymal cells. The induced collagenolytic activity in nuclear fraction is also interpreted as being due to the activity in mesenchymal cells, contaminated in this fraction. At present the collagenolytic activity and presumably the collagenase activity induced by cortisone administration seems to be partly responsible for the reduction of hepatic collagens.



Intracellular distribution of collagenolytic activity towards acid soluble collagen in rat liver with or without contisone administration; Nuc, Mit, Lys, Mic and Sup denote nuclear, mitochondrial, lysosomal, microsomal and supernatant fraction.

As indicated by the present investigation, insoluble collagen does not seem to be sensitive to cortisone, so that the cortisone effect may be mainly limited to the metabolism of soluble collagen, similar to the conditions of carbon tetrachloride poisoning[6,11]. In fact, corticosteroid in the quantity administered in the present study did not increase in vivo urinary hydroxyproline excretion in rats[15], suggesting that the insoluble collagen, which occupies 90% of body collagen, is really metabolically inert. Finally the mode of action of cortisone on hepatic collagens could be explained on the basis of: 1. an inhibition of the anabolism in fibroblasts; 2. an induction of the catabolism by lysosomal collagenolytic activity, resulting in a reduced hepatic content of collagen.

Zusammenfassung. Nach Cortison-Behandlung wird sowohl der Gehalt als auch die spezifische Aktivität des neutralen löslichen Kollagens in der Rattenleber vermindert. Die kollagenolytische Aktivität für das lösliche Kollagen vermehrt sich hingegen und zwar trotzdem die Aktivität für unlösliches Kollagen verändert blieb.

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Proteolytische Enzyme aus dem Darmtrakt der Hornisse (*Vespa orientalis* F.)

Die Evolution der Organismen, die bisher weitgehend aus anatomisch-morphologischer Sicht erklärt worden ist, beruht letztlich auf Mutationen, wobei morphologische Erscheinungsformen nur der sichtbare Ausdruck einer Evolution im molekularen Bereich sind. Für vergleichende Untersuchungen dieser Art sind Proteine sehr günstig, wie die Untersuchungen über das Hämoglobin und das Cytochrom C zeigen[1,2]. In letzter Zeit wurden ebenfalls Proteasen in diese Fragestellung einbezogen, weil sie wegen ihrer notwendigen Einstellung auf verschiedene physiologische Situationen für den evolutionären Gedanken geeignet erscheinen[3,4].

Lyophilisierte Mitteldärme von orientalischen Hornissen-Larven wurden in 0,01 *M* *Tris*/HCl-Puffer, pH 8,0 aufgenommen und durch Zentrifugation von gröberen Partikeln befreit. Im so erhaltenen Rohextrakt liess sich mit Casein als Substrat eine proteolytische Aktivität nachweisen, die durch die Hydrolyse von spezifischen Estern bereits auf zwei Endopeptidasen hindeutete. Im weiteren Verlauf der Reinigung über einen Anionenaustauscher (DEAE Sephadex A-50) mit 0,01 *M* *Tris*/HCl-Puffer, pH 8,0) und anschliessender Gelfiltration (Sephadex G 75 mit NH_4HCO_3-Puffer, 0,1 *M*, pH 8,0) konnten dann zwei Fraktionen isoliert werden, wovon eine das chymotrypsinspezifische Substrat N-Acetyltyrosinäthylester (ATEE) und die andere das trypsin-spezifische Substrat Nα-Benzoylarginin-äthylester (BAEE) spaltete. Die Anreicherung in bezug auf die spezifische Aktivität betrug für die BAEE-Fraktion 9fach, für die ATEE-Fraktion 14 fach. Bei der anschliessenden elektrophoretischen Prüfung auf CA-Membranfolien (Veronal-Acetat-Puffer-0,036 *M*, pH 8,6) zeigte sich ein unterschiedliches Verhalten dieser Fraktionen untereinander als auch im Vergleich mit Trypsin und Chymotrypsin. Die ATEE-spaltende Fraktion wandert wie das Chymotrypsin in kathodischer Richtung, nur läuft sie nicht so weit, während die BAEE-spaltende Fraktion in umgekehrte, anodische Richtung wandert. Diese Wanderungsunterschiede stimmen auch mit dem Befund überein, dass sich das ATEE-spaltende Enzym nach Auftragen auf die Anionen-Austauscher-Säule im Durchlauf befindet, während das BAEE-spaltende Enzym daran festgebunden wird und sich erst mit Hilfe eines NaCl-Gradienten abtrennen lässt. Diese teilweise deutlichen Ladungsunterschiede lassen auf eine Differenz in der Aminosäurenzusammensetzung schliessen, die zwischen der ATEE-spaltenden Protease und dem Chymotrypsin weniger deutlich ist als zwischen der BAEE-spaltenden Protease und dem Trypsin. Dass die einzelnen Banden, die mit Coomassie-Blau als Proteine identifiziert werden konnten, wirklich die aktiven Enzyme waren, wurde in einem Agarose-Sandwichverfahren mit Acetylphenylalanin-naphthylester (APNE) und Benzoylargininnaphthylamid (BANA) in Verbindung mit Diazoblau nachgewiesen[5].

Interessanterweise lässt sich in einem weiteren Versuch mit Königinnen-Larven keine BAEE-hydrolysierende