DECREASED PROTEIN CATABOLISM DURING STIMULATED GROWTH

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1. Introduction

Compensatory renal hypertrophy, regenerating liver, and the isoproterenol stimulated salivary gland are useful models for studying the changes in protein metabolism during accelerated growth. Previous experiments suggested that the protein gain in these organs was accomplished by an increased rate of protein synthesis [1], although a reduction in the rate of protein degradation could account for the increased protein content. Reduced protein catabolism has previously been shown to occur during bacterial growth [2], muscle hypertrophy [3], liver regeneration [4], and in numerous other systems [5]. This report shows that a decrease in degradation of pre-existing proteins contributes significantly to net protein gain during renal hypertrophy, liver regeneration, and isoproterenol-stimulated parotid growth. Before stimulation of growth, proteins were labelled with arginine (guanido-^{14}C) and the mean half-life was determined by following the loss of protein radioactivity. The half-life of renal proteins increased 66% during compensatory renal hypertrophy. Following partial hepatectomy, the half-life of hepatic proteins increased 71%. Daily injections of isoproterenol increased the mean half-life of parotid proteins 84% while cardiac protein half-life was not changed. This is the first report of the significance of protein degradation during renal hypertrophy and isoproterenol-stimulated parotid growth.

2. Materials and methods

Charles River male mice (6–8 weeks old, 30–35 gm) were maintained in a 12 hr light-12 hr dark cycle for one week; Purina lab chow and water were given ad lib. Mice received injections of L-arginine (guanido-^{14}C) (Schwarz/Mann, 46 mCi/mmole) 36–48 hrs before growth stimulation. The isotope injections were given subcutaneously so that isotope distribution was more uniform among the several tissues. Partial hepatectomy was performed as described by Higgins and Anderson [6]; uninephrectomy was performed as previously described [7]. Isoproterenol (Mann Research Laboratories) was suspended in sesame seed oil (25 μg/ 0.2 ml) and 0.2 ml was injected subcutaneously each afternoon. Control mice received only sesame seed oil. Tissue samples were frozen and stored (-25°C) at least two days. After all tissues were collected, they were processed together on the same day. Samples were homogenized in 5% citric acid–0.25 M sucrose and aliquots were taken for estimation of protein, nucleic acids and radioactivity. Protein concentration was assessed by the method of Lowry, et al. [8]. DNA and RNA fractions were prepared and analyzed as described by Scott, et al. [9] as modified by Hinrichs, et al. [10].

3. Results and discussion

Fig. 1A shows the loss of radioactivity in the remaining mouse kidney after uninephrectomy or sham operation. Since the DNA content does not change during renal growth [7], this value represents protein radioactivity per cell. The mean half-life of the kidney proteins is 2.9 days for sham mice and 4.8 days for the uninephrectomized mice (table 1).

Treatment of mice with daily doses of isoproterenol

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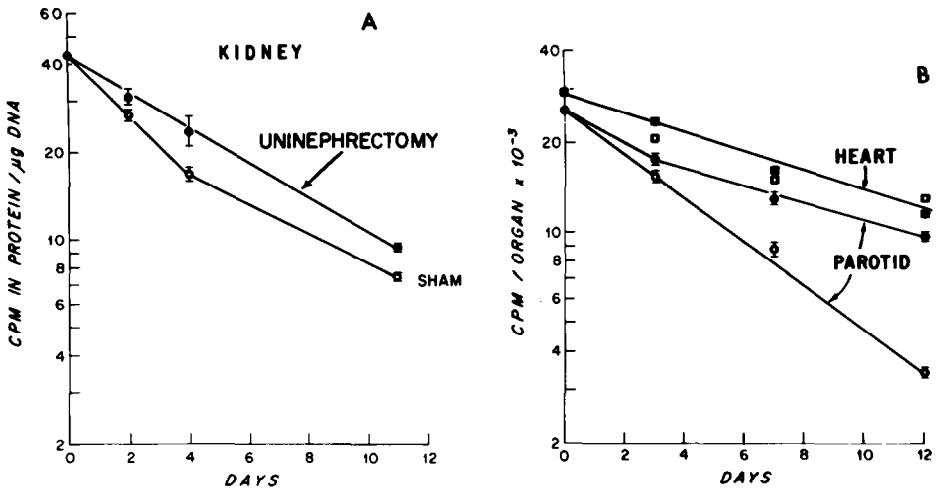

Fig. 1. A) Mice were injected with 20 μcuries of arginine (guanido-^{14}C). 48 hr later all animals were subjected to either uninephrectomy (●—●—●), or sham operation (○—○—○). The kidneys removed from uninephrectomized mice were used to determine the zero time point. At the time intervals indicated, mice were killed and kidneys were taken and frozen at −25°C. DNA and protein fractions were prepared as described in the text. Each point represents the mean and standard error for three mice. B) Mice were injected subcutaneously with 20 μcuries of arginine (guanido-^{14}C) 36 hr prior to receiving their first injection of isoproterenol. At 24 hr intervals, mice were injected with 25 μg of isoproterenol (●—●—●, ■—■—■) or vehicle only (○—○—○, □—□—□). At the indicated time intervals, mice were killed and parotid glands and hearts were taken and frozen at −25°C. Since the parotid glands grew extensively over the time period studied, results are presented as radioactivity in protein per organ. Each point represents the mean and standard error for four mice.

Table 1
Growth and protein degradation rates in liver, kidney, heart and parotid

		$T_{½}$	Per cent of control $T_{½}$	Per cent of control wet weight
Liver	Sham operation	3.8		
	Partial hepatectomy	6.5	171	N.D.
Kidney	Sham operation	2.9		
	Uninephrectomy	4.8	166	139
Parotid	Control	3.8		
	Isoproterenol	7.0	184	276
Heart	Control	8.0		
	Isoproterenol	8.0	100	104

The mean half-life of proteins isolated from non-growing and growing tissues was determined as described in the text. As an overall index of growth, the percent change in the wet weight of kidney, parotid, and heart, over the 12 day period is indicated.

resulted in an increase in growth and average cell protein half-life of the parotid gland but no change in growth or half-life of heart proteins. Proteins from heart and parotid gland of the control group had mean half-lives of 8.0 and 3.8 days, respectively (table 1). Heart proteins from isoproterenol treated animals had a mean half-life of 8.0 days. The parotid proteins from the isoproterenol treated animals had a mean half-life of 7 days with a 13 day half-life for the last 9 days of treatment (fig. 1B). The two component decay curve followed the parotid growth pattern. The data in fig. 1B are expressed as protein radioactivity

per organ since parotid DNA, RNA, protein, and wet weight all increased with isoproterenol treatment.

The results in table 1 for regenerating liver are similar to those reported by Scornik [4]. The half-life of liver proteins from sham operated animals was 3.8 days. Values reported from other laboratories for protein half-life in normal liver have been 3.3–4.0 days [11]. Our results indicate that partial hepatectomy increases the mean half-life of liver proteins to 6.5 days.

Although some of the observed increase in protein half-life could be accounted for by increased reutilization of arginine, it appears more likely that the changes seen here are largely the result of decreased catabolism. Investigations of protein synthesis after uninephrectomy [12] or in isoproterenol-stimulated salivary glands [13] have found increases too small or occurring too late to account for the increase in protein content.

Tomashefsky and Tannenbaum [12] have suggested that protein accretion during compensatory renal hypertrophy is due to an increased rate of protein synthesis with no significant changes in protein degradation. They calculated the mean half-life of rat kidney proteins without compensating for the net protein gain (between 28% and 57% in their system). If their data are recalculated using radioactivity in total protein per total DNA, a significant decrease in protein catabolism is evident. Furthermore, Goldberg [3] has reported unpublished data which suggest a decreased degradation of kidney proteins during compensatory hypertrophy.

An estimate of the relative contributions of synthesis and degradation can be made using a simplified model as described by Schimke [14] ($P = (P_o - \frac{S}{k})(e^{-kt} + \frac{S}{k})$ [14][1]. Our calculations indicated greater than 95% of the net protein gain under stimulated growth conditions in kidney could be accounted for by a decrease in protein catabolism*. All these examples signal the importance of the regulation of protein catabolism in organ growth.

* Rates of the protein synthesis in kidney. Calculations are based on a 29% increase in protein at 4 days after uninephrectomy. S is the zero-order synthetic rate (mg protein/μg DNA/day); P_0 is protein content at steady state (mg protein/μg DNA); k is the first order degradation constant. Sham values are $t_{1/2} = 2.90$; $k = 0.24$; $S = 0.24\ P_0$. Uninephrectomy values are $t_{1/2} = 4.8$, $k = 0.14$; $S = 0.23\ P_0$.

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References

[1] Bucher, N. L. R. and Malt, R. A. (1971) Regeneration of Liver and Kidney, Little, Brown and Co., Boston; Tsukada, K., T. Moriyama, T. Umeda and Lieberman, I. (1968) J. Biol. Chem. 243, 1160–1165; Bignall, M. C., Elebute, O. and Lotspeich, W. D. (1968) Am. J. Physiol. 215, 289–295; Coe, F. L. and Korty, P. R. (1967) Am. J. Physiol. 213, 1585–1589; Tomashefsky, P. and Tannenbaum, M. (1969) Lab. Invest. 21, 358–364; Ekfors, T. and Barka, T. (1971) Lab. Invest. 25, 287–290; Sasaki, T., Litwack, G. and Baserga, R. (1969) J. Biol. Chem. 244, 4831–4837.

[2] Mandelstam, J. (1960) Bacteriol. Rev. 24, 289–308.

[3] Goldberg, A. L. (1971) in: Cardiac Hypertrophy (Alpert, N. R., ed.), pp. 301–314) Academic Press, New York.

[4] Scornik, O. A. (1972) Biochem. Biophys. Res. Commun. 47, 1063–1066.

[5] Goss, R. J. (1970) in: Adv. Cell Biol. pp. 233–296, Appleton-Century-Crofts, New York; Schimke, R. T. and Doyle, D. (1970) in Annual Review Biochem. 39, 929–976; Mammalian Protein Metabolism IV (1970), chapters 30, 32 and 39 (Munro, H. N., ed.); Recheigl, M. (1971) Enzyme Synthesis and Degradation in Mammalian Systems, University Park Press, Baltimore; Pine, M. J. (1972) Ann. Rev. Microbiol. 26, 103–126; Thatcher, J. D. (1971) in: Cellular and Molecular Renewal in the Mammalian Body pp. 153–219, Academic Press, New York.

[6] Higgins, G. M. and Anderson, R. M. (1931) Arch. Path. 12, 186–202.

[7] Malt, R. A. and Lemaitre, D. A. (1968) Am. J. Physiol. 214, 1041–1047.

[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.

[9] Scott, J. F., Fraccastoro, A. P. and Taft, E. B. (1956) J. Histochem. Cytochem. 4, 1–10.

[10] Hinrichs, H. R., Peterson, R. D. and Baserga, R. (1964) Arch. Pathol. 78, 245–253.

[11] Schimke, R. T., Ganschow, R., Doyle, D. and Arias, I. M. (1968) Fed. Proc. 27, 1223–1230; Kuehl, L. and Sumsion, E. N. (1970) J. Biol. Chem. 245, 6616–6623

[12] Tomashefsky, P. and Tannenbaum, M. (1970) Lab. Invest. 23, 190–195.

[13] Ekfors, T. and Barka, T. (1971) Lab. Invest. 24, 197–202.

[14] Schimke, R. T. (1967) Natl. Cancer Inst. Monograph 27, 301–315.