SYNTHESIS AND DEGRADATION OF MICROVILLAR PROTEIN OF KIDNEY IN ADULT RATS

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1. Introduction

Cells devoted to absorptive function in the small intestine (enterocytes) and kidney cortex (proximal tubules) have luminal membranes equipped with microvilli or brush borders of a typical morphological structure. Recently developed procedures for the isolation of kidney brush border membranes from rat have enabled some characterization of their biochemical properties [1]. Structure and function of these membranes are obviously related to the dynamics of their synthesis and degradation. Quirk et al. [2] have studied the protein turnover of the brush borders isolated from rabbit kidney. Herein, we summarize results of our experiments where the rates of synthesis and degradation of brush border protein and total homogenate of rat kidney cortex were studied by pulse labeling whith [^{14}C] leucine or L-arginine ([^{14}C] guanido). Our results indicate that amino acids are incorporated into brush border protein more rapidly than homogenate protein and that the turnover rate of membrane protein is greater than homogenate protein, findings which differ considerably from the situation in rabbit kidney [2].

2. Methods

2.1. *Animals*

Experiments were performed using male Sprague–Dawley rats (Charles River, Mass.) weighing 250–300 g. Rats were purchased at the age of 5 weeks and were used 3–5 weeks after acclimatization to their new environment. Food and water intake was not restricted any time during the experiments.

2.2. *Pulse labeling*

Labeled amino acids (10 μCi per rat) were injected i.p. (20 μCi/ml in 0.9% NaCl) in the morning hours and rats were then returned to their cages. The injected animals were sacrificed from 15 min to 216 hr after the radioactive pulse. In chase experiments, rats received subcutaneous injections of unlabeled leucine (33 mg/100 g body weight) or arginine (12 mg/100 g body weight) dissolved in 0.9% NaCl and given in a volume of 3 ml per rat 12–15 hr after the ^{14}C pulse and every 24 hr thereafter.

2.3. *Preparation of brush border fractions*

Rats were decapitated by guillotine. The microvillar fraction from the kidney cortex was isolated by the modified method of Kinne and Kinne–Saffran [1, 3]. The increase of specific activity of alkaline phosphatase [4] in kidney brush border over homogenate was 6-fold in STE buffer (0.25 M sucrose, 0.01 M triethanolamine hydrochloride, 0.005 M EDTA, pH 7.6) which agrees with data given by Kinne (personal communication). Light and electron microscopic control of brush border fractions indicated a reproducibly good quality of microvillar preparations.

2.4. *Analysis of radioactivity*

Analysis of radioactivity was performed in a slightly modified form according to the method of Mans and Novelli [5]. To one volume of homogenate or brush border fraction containing 0.5–0.9 mg of protein as assayed by the method of Lowry et al. [6] four volumes of 20% trichloroacetic acid were added. This mixture was heated at 90°C for 15 min and then filtered through glass fiber filter papers (Reeve Angel #934AH, Clifton, N.J., USA) in a Hoeffer Scientific filtration apparatus (San Francisco, Calif., USA). Precipitates were subsequently washed three times with 10% TCA, once with absolute alcohol, once with alcohol:ether (1:1), and fianlly with anhydrous ether. The filters were air dried, placed in 2 ml dilute Liquifluor (Packard Instruments, Downers Grove, Ill., USA), and assayed for radioactivity in a Packard Tricarb Scintillation Spectrometer. The efficiency of counting was 88% as determined by using the external automatic standard.

2.5. *Evaluation of data*

Radioactivity was expressed as cpm/mg of protein. In order to compare results from animals differing in weight from various experiments the cpm/mg protein value was further divided by the dose (in μCi) given per 100 g body weight (e.g., rats weighing 275 g receiving 10 μCi; the cpm/mg protein value was divided by 3.63). The data of experiments where the half-lives were determined were plotted on a semi-logarithmic scale. The line was fitted by the method of least squares. This calculation as well as determination of correlation coefficient was performed on the Monroe 1775 computer. The Student's *t*-test was used as well as the method described by Goldstein [7] for determination of statistical significance between slopes of two lines.

2.6. *Chemicals*

Analytical grade reagents were used throughout. Uniformly labeled [^{14}C]-L-leucine (262 mCi/m mole) and L-arginine ([^{14}C]guanido) (4.58 mCi/m mole) were purchased from New England Nuclear Corp. (Boston, Mass., USA).

3. Results

3.1. *Incorporation of [^{14}C] leucine into homogenates and brush borders of kidney cortex*

Results obtained in *kidney cortex* are demonstrated in figs. 1 and 2. The cortex homogenate (fig. 1A) reached its peak specific activity between 2 and 4 hr after which a decrease was observed (fig. 2). The maximum specific activity for the brush border fraction was obtained between 1 and 2 hr and seen to decrease after 4 hr (fig. 1B and fig. 2).

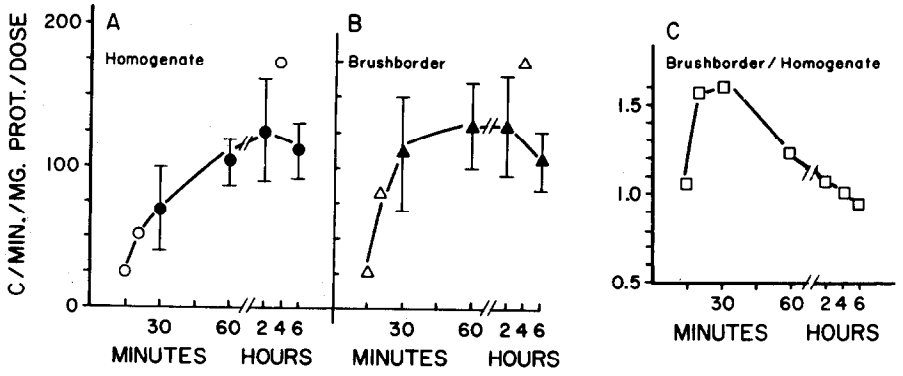

Fig. 1. Incorporation of [^{14}C] leucine into proteins of homogenate of kidney cortex (A) and of brush borders (B). Full symbols denote means of 3–7 determinations, short vertical lines denote 2 S.E.M. Open symbols: single determination. For definition of units see Methods. (1C) Ratio of specific activity of homogenates. Statistical evaluation: in the kidney cortex the difference between the ratios of the 30′ values and the combined 2–4 hr values – $p < 0.01$.

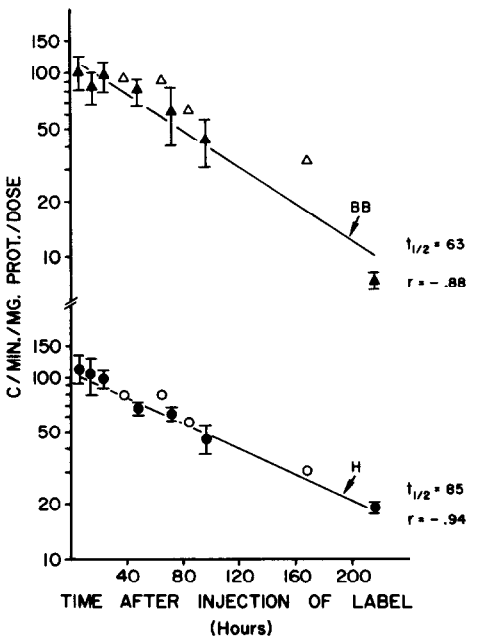

Fig. 2. Turnover of ^{14}C-labeled proteins in brush borders (△, ▲) and homogenate of kidney cortex (○, ●). Full symbols denote mean of several determinations, short vertical lines denote 2 S.E.M.; open symbols denote individual values from different experiments, where only one time point was available. The regression lines were calculated from all individual values by the least squares method – total number of points originating from 39 animals – and the difference in slopes of the two lines was found to be significant ($p < 0.05$) using the formula for t-test given by Goldstein [13].

In order to demonstrate the different pattern of [^{14}C] leucine incorporation into the microvilli and homogenate of the kidney cortex, the values have been expressed also as the ratio of specific activity of the brush borders to the homogenates. This is presented in fig. 1C which shows the ratio to be 1.0 or above from the first time point measured (15 min).

3.2. *Loss of radioactivity after pulse labeling with ^{14}C amino acids*

Fig. 2 demonstrates the decrease of specific activity in the total homogenate of kidney cortex and brush border fraction in animals injected with [^{14}C] leucine. Data from rats sacrificed 6 hr or more after injection were used. The half-life for the homogenate was longer ($t_{1/2}$ = 85 hr; $r = -0.94$) than the half-life of the brush border fraction ($t_{1/2}$ = 63 hr; $r = -0.88$). Neither exclusion of the 216 hr time points nor the correction for dose/g body weight changed this relationship substantially. The longer time points allowed calculation of $t_{1/2}$ over a range equal to at least two half-lives. Injection of unlabeled leucine (see Methods for s.c. chase) did not change these values. Also the use of another amino acid – L-arginine ([^{14}C] guanido) – resulted in lower incorporation but gave similar results; half-life of the homogenate was again longer than the brush border fraction.

4. Discussion

Interpretation of turnover studies may be complicated by re-utilization of the label employed resulting in apparently longer half-lives of the proteins [8]. Half-lives of different organs and cell fractions could reflect different degrees and rates of re-utilization. Thus, we have attempted to circumvent this problem by keeping the animals on a normal feeding regimen to dilute the precursor pool and by using two different amino acids including a 'chase' in some experiments. The lack of effect of 'chase' by unlabeled amino acids on the half-lives does not completely exclude the possibility of preferential re-utilization of labeled over unlabeled amino acids which would reside in different pools. Recently, Mortimore et al. [9] have shown the existence of two distinct intracellular valine pools in perfused rat liver in situ; one was independent of external valine and appeared to contain free valine arising from proteolysis whereas the second one equilibrated easily with external valine and was in continuity with sites of protein synthesis.

Quirk et al. [2] have recently studied the turnover of kidney brush border protein in another species – rabbit using [^{3}H] lysine and [^{14}C] glucosamine. Maximum radioactivity in the whole cortex homogenate occurred at 5 hr and in the brush border at 15–20 hr. In our experiments with rats using [^{14}C]-leucine maximum incorporation was observed earlier, i.e., homogenate 2–4 hr and brush border 1–2 hr. In experiments of Quirk et al. [2] the disappearance of label from the rabbit homogenate protein was exponential while that in the brush border protein appeared biphasic. However, their data were from points derived from single animals which would not compensate for individual variation. Our data in rat showed

an exponential decline of protein incorporated radioactivity in both whole cortex homogenate and brush border which was faster than in the rabbit. The present observations are in agreement with those previously published in whole rat kidney homogenate using [^{35}S] methionine [10]. The differences observed between the data from rabbit and rat may be species differences in general or an effect of the handling of different amino acids used to study turnover.

Our experiments have shown that the half-lives of homogenate and microvillar proteins of kidney cortex are longer than the previously published half-lives of homogenate and microvillar proteins of jejunal mucosa [11, 12]. This may reflect differences in cell turnover rate between jejunum (cell life – 2 days) and kidney (very little if any cell turnover [13]). Our data show that in kidney cortex the half-lives of the microvillar proteins are shorter than those of the homogenate proteins indicating that microvilli proteins are formed, rebuilt or renewed faster than the total cell protein. A similar conclusion was drawn by James et al. [12] for the intestine. In the kidney cortex, the increase of specific activity in the brush border precedes that in the homogenate. Since the opposite is true for jejunum, James et al. [12] have proposed that the transfer of labeled protein molecules from the intestinal cell (microsomes), where they are synthesized, to the brush borders takes time. In the kidney cortex, however, either the precursor proteins are preferentially transferred to the membrane or protein synthesis takes place either in the kidney brush border or in close proximity to it. This remains open to further studies as does the determination of the synthesis and turnover of individual proteins located in the brush border.

Acknowledgements

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