Selective Postnatal Development of Na,K-Activated-Adenosinetriphosphatase in Rabbit Kidneys (35202)

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Na,K-activated, Mg-dependent-adenosinetriphosphatase (ATPase), a membrane-bound enzyme which appears to be involved in the transport of sodium and potassium ions across cell membranes, has received wide attention since its discovery by Skou in 1957 (1). Evidence in recent years suggests the participation of this enzyme system in the tubular reabsorption of sodium in the kidney (2) and also possibly in the renal secretion of *p*-aminohippuric acid (PAH) (3). Progressive increases in renal functional capacity with increasing age during the perinatal period have been suggested (4); tubular transport processes undergoing such maturation include sodium reabsorption (5) and PAH secretion (6–9). In addition, the tubular reabsorption of amino acids, a process which exhibits sodium dependency with possible involvement of Na,K-activated ATPase (10), is less functional in immature kidneys (11, 12). This study was therefore designed to determine if the levels of kidney ATPase activity, especially Na,K-activated-ATPase, in newborn and young rabbits differ from those in the adult.

Materials and Methods. Whole kidney from rabbits ranging in age from 1 day to 12 weeks (adult) were removed immediately following sacrifice by cervical dislocation. The tissues of animals within each age group were pooled and homogenized in 8 vol of ice-cold 0.25 *M* sucrose containing 5 m*M* EDTA, 0.1% deoxycholic acid, and 30 m*M* Tris at pH 7.5. All further procedures were performed at 0–4°. A heavy microsomal fraction, prepared by centrifugation (35,000*g* for 30 min) of the 12,000*g*, 30 min supernate, resuspended in the homogenizing medium and stored for 2 days at 0°, served as the source of ATPase activity in this study. NaKMg-ATPase activity was determined using a mixture containing 3 m*M* Tris ATP, 100 m*M* NaCl, 15 m*M* KCl, 3 m*M* $MgCl_2$, and 30 m*M* Tris at pH 7.5 with incubation at 37° for 15 min. Mg-ATPase activity was measured in the presence of 115 m*M* choline in place of the omitted Na and K. The addition of 0.1 m*M* ouabain, an alternative means for Mg-ATPase assay in the presence of Na and K, also was used throughout the study. This method provided virtually the same results as the NaK omission/choline method. NaK-ATPase activity was calculated as the difference between NaKMg-ATPase and Mg-ATPase values. The reaction was determined after incubation by the addition of perchloric acid. After centrifugation, liberated inorganic phosphate in the supernate was measured by the Fiske and SubbaRow method (13) with correction by means of appropriate blanks for nonenzymatic hydrolysis. Microsomal protein was determined by a modified biuret method (14) and the method of Lowry *et al.* (15) using crystalline bovine albumin as the standard. Enzymatic activity was expressed as micromoles of inorganic phosphate liberated per milligram of protein in 15 min. In all experiments, the preparation of microsomes and enzyme assays (48 hr later) were performed simultaneously for all age groups processed on a given day.

Results. Values for the various components of ATPase activity at intervals of increasing age are presented in Fig. 1. Total Na,K-activated, Mg-dependent-ATPase activity increased gradually with age from newborn to adult. Mg-dependent-ATPase, however, re-

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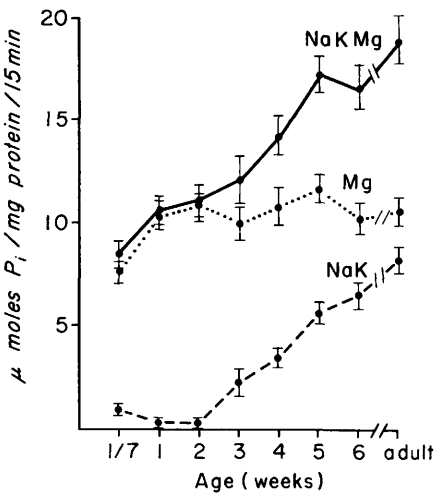

FIG. 1. Development in rabbit kidneys of the Na,K-activated, Mg-dependent-ATPase enzyme system as a function of age. Each point represents the mean ± 1 SE for a set of either four or five independent observations on pooled tissues from one to eight animals each. (—) NaKMg-ATPase; (...) Mg-ATPase; (---) NaK-ATPase.

mained essentially unchanged and equivalent to the adult level at all ages. Na,K-activated-ATPase was nearly absent at birth and at 5 weeks of age approached levels comparable to those of the adult. The observed increase in NaKMg-ATPase was accounted for by this selective increase in the NaK-activated component of ATPase activity.

In sheep, we found a similar pattern of ATPase development with age by comparing enzyme levels in fetal and maternal kidneys near term. In a series of four independent fetal/maternal pairings, total NaKMg-ATPase in sheep fetal kidneys was only 65% of the maternal level. Whereas fetal Mg-ATPase (76% of maternal) did not differ from adult activity at a 0.05 confidence level, the NaK-activated component of fetal ATPase (39% of maternal) increased signifiantly with age.

Discussion. Beyth and Gutman (16) recently published a preliminary note on renal ATPase activity in rabbits of four different age groups; fetal, day old, 10 days old, and adult. Their observations and reported values correspond in general to those in the present study. However, their adult value for NaK-ATPase is only about one-fifth of that we have reported, and their value for Mg-dependent-ATPase is significantly less in the newborn than in the adult. Therefore, these investigators missed the gradual increase in the NaK-ATPase and concluded that Mg-ATPase increased progressively during postnatal development. It is unfortunate that only three postnatal age groups were included in their study.

Hirsch and Hook (9) have recently studied PAH renal secretion by rabbits at birth and at 1, 2, and 4 weeks of age. These investigators reported that the slice/medium ratio of PAH increased minimally during the first 2 weeks of life and reached a peak at 4 weeks of age. These physiological observations correlate well with the biochemical studies presented here. During the first 2 weeks of life, there is only minimal organic acid transport and very low levels of NaK-ATPase. Thus, it appears the rabbit's ability to secrete PAH is less functional early in life when NaK-ATPase values are low and increases toward adult levels during the same time that NaK-ATPase activity is increasing. In contrast, the Mg-dependent-ATPase remains constant during the maturation of the renal PAH secretory mechanism.

If one accepts the proposition of Csaky (17) that Na,K-activated-ATPase may be an integral part of all sodium-dependent active transport processes, then our data may account for a deficient capacity in renal transport mechanisms for amino acids, PAH, and sodium ion during the perinatal period.

Summary. Renal ATPase activity was measured in rabbits of different ages. Total Na,K-activated, Mg-dependent-ATPase activity increased gradually with age from newborn to adult. Mg-dependent-ATPase, however, remained essentially unchanged and equivalent to the adult level during development. Na,K-activated-ATPase was nearly absent at birth and at 5 weeks of age approached levels comparable to those of the adult. The increase in total ATPase activity was due to this selective increase in the Na,K-activated component. The development of Na,K-activated-ATPase activity correlates well with the reported maturation of renal

transport mechanisms for amino acids, *p*-aminohippuric acid, and sodium ion during the perinatal period.

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