Accelerated Transport of α-Aminoisobutyric Acid in Kidney During Compensatory Hypertrophy[1] (37082)

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Estimates of protein synthesis during the early phase of compensatory hypertrophy of the rat kidney have not been uniform. Increments of 3 to 11% have been recorded at the end of the first day (1–4). In slices taken from kidneys during that time the kinetics of labeling with radioactive leucine have been described as having either a unimodal or bimodal peak (3, 5, 6); however, in a microsomal fraction no increase was found (7).

Although some of the variance may have been produced by the different strains of rats employed, analyses of the kinetics of protein labeling have probably also been influenced by differences in the specific activity of the immediate-precursor pool. The present study is concerned with the concentration of a nonmetabolizable amino acid, α-aminoisobutyric acid (AIB), in mouse kidney during the first week of compensatory hypertrophy. This amino acid is a model compound for the active transport of short-chain polar amino acids (8, 9). The results show a significant increase in the renal concentration of AIB, which reaches its peak 48 hr after contralateral nephrectomy.

Methods. Male Charles River mice (31–35 g) were maintained under constant illumination and given Purina rat chow and water freely. They were allocated randomly to one of three groups: (1) right nephrectomy, (2) sham nephrectomy, (3) unoperated control. Right nephrectomy was performed under light ether anesthesia through the flank, sparing the adrenal. In sham nephrectomies the right kidney was exposed, but not touched.

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Sixty minutes before sacrifice each mouse received an intraperitoneal injection of 0.30 ml of an isotonic saline solution of 20 μCi/kg of 1-^{14}C-α-aminoisobutyric acid (20.6 mCi/mmole, New England Nuclear Corp., Boston). Thirty minutes later each received 0.30 ml of an isotonic saline solution of 188 μCi/kg of ^{3}H-methoxy-inulin (MOI) (94.2 mCi/g, New England Nuclear).

From heparinized blood obtained at decapitation, 0.1 ml of plasma dissolved in 1.5 ml Soluene 100 (Packard Instrument Co., Downers Grove, IL) was counted in 10 ml of scintillator (3 parts toluene:2 parts ethylene glycol monomethyl ether) plus 4.5 g/liter Omnifluor (New England Nuclear) at 82% efficiency for ^{14}C and 30% efficiency for ^{3}H.

At intervals to 1 wk after right nephrectomy the left kidney was removed, decapsulated, and serially sectioned. After digestion of ~ 120 mg sections in 2.0 ml of 1 *N* NaOH at 55° for 90 min, 0.1 ml samples were counted in 10 ml of scintillator at 83% efficiency for ^{14}C and 19% efficiency for ^{3}H. All samples of plasma and tissue were corrected to 100% efficiency. Protein contents were estimated with the Lowry *et al.* method (10).

Renal uptake of AIB was calculated as the ratio of dpm in kidney compared with those in plasma, and the volume of the renal extracellular space was similarly calculated from MOI ratios.

Results. As in experiments by others (11, 12), a labeling period of 1 hr was used with AIB because experiments with our system also showed a constant plasma level of AIB 0.5 hour after injection and a stable ratio of renal AIB/plasma AIB at 1 hr, rising

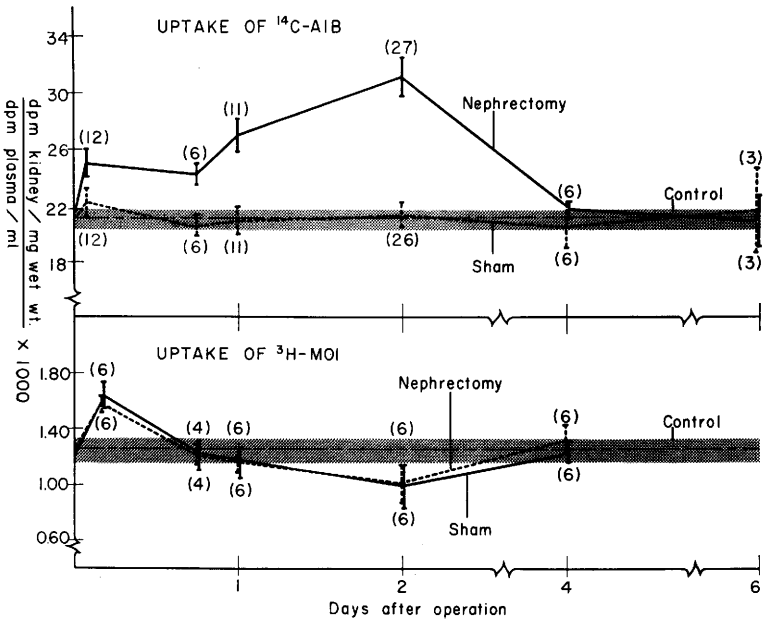

FIG. 1. Concentration of AIB and MOI after nephrectomy and sham nephrectomy.

only slowly to 3 hr. One hour after injection in control mice, the renal level of AIB was 493,000 ± 9600 dpm/mg wet weight (mean ± SE, $N = 14$); the plasma level was 23,100 ± 800 dpm/ml; the average of the individual ratios of uptake was 21.1 ± 0.5.

One-half hour was selected as the labeling period with MOI because this interval was close to the maximal period of linear incorporation, as reported (11, 12). The ratio of MOI uptake in control kidneys was 126 ± 0.36 (mean ± SE, $N = 8$).

After an increase of uncertain significance ($p > 0.05$) 4 hr after nephrectomy, from 18 to 48 hr after unilateral nephrectomy compared with sham nephrectomy there was an increase in the renal concentration of AIB, reaching an increase of 25% ($p < 0.01$) at 24 hr and an increase of 49% ($p < 0.01$) at 48 hr (Fig. 1). Corresponding values normalized for renal protein content were 29 and 63%, respectively. After the maximum at 48 hr, the ratio returned to normal by 96 hr. The uptake of AIB into liver of all three experimental groups was steady at a ratio of 7.7 ± 0.1 (mean ± SE) throughout.

Except at 4 hr after unilateral nephrectomy when the ratio of MOI uptake was increased about 36% above controls in both nephrectomized and sham-nephrectomized mice ($p < 0.05$), the levels were similar to those of control mice (Fig. 1).

Discussion. As judged from the uptake of MOI, both the sham-nephrectomized mice and the nephrectomized mice showed an increase in extracellular fluid volume 4 hr after operation. Since this increase is apparently a nonspecific effect of an operation, the elevated tissue levels of AIB measured somewhat earlier probably reflect the increased extracellular volume rather than accelerated concentration of AIB. After the eighteenth postoperative hour, however, when the levels of MOI in the sham-nephrectomized mice and the nephrectomized mice were not significantly different from those of control mice, the elevated AIB levels in the remaining kidneys of unilaterally nephrectomized mice would seem to reflect active concentration of AIB.

This accelerated concentration of a model short-chain polar amino acid implies that application of such a radioactive compound as a label for protein during that interval must also have concurrent corrections for the specific activity of the immediate precursor

pool before the kinetics of labeling can in any way be held to reflect the kinetics of protein synthesis. No matter whether the amino acid pool in the remaining kidney decreases (13) or remains stable (unpublished data) after contralateral nephrectomy, accelerated amino acid transport would increase the specific activity of the precursor pool. The situation is further complicated by the likelihood that the amino acid pool in kidney is not in free equilibrium, but rather is compartmented (14).

Compared with other measurements of AIB uptake in normal rat kidney and liver, the levels of AIB in mouse kidney and liver reported here were 30% lower (15), probably because of different methods of preparing the samples and of expressing the data. The ratio of the levels of kidney compared with the levels of liver was also 45% lower. The concentration of AIB after 8 hr of compensatory hypertrophy of the kidney is slower than the increases observed within 2 hr of stimulation of levator ani muscle and within 4 hr of experimentally produced hypertrophy of skeletal muscle, but considerably faster than the maximal increase found in myocardium 3.5 days after aortic banding (11, 12, 16).

Summary. The renal concentration of α-aminoisobutyric acid, corrected for extravascular fluid volume, increases at least 50% by the second day after contralateral nephrectomy and returns to normal in the next 2 days. The data suggest the necessity of correcting the labeling kinetics of renal protein during compensatory renal hypertrophy for changes in the specific activity of the amino acid precursor pool.

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