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Nonsaturable Amino Acid Reabsorption in Kidneys of Normal and Mercury-Poisoned Rabbits (42802)

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Abstract. At high plasma concentrations, a high-capacity, low-affinity or nonsaturable flux (J_{hc}) accounts for a residual fractional reabsorption of cycloleucine, aspartate, and AIB of approximately 50% of the filtered load in rabbits; J_{hc} in micromoles per milliliter glomerular filtrate is reduced in Hg-poisoned animals. The nonsaturable flux of cycloleucine is characterized by a transepithelial transit time (TET) of approximately 2 min in control animals; it was consistently much longer in Hg-poisoned animals. The clearance ratio of creatinine/inulin averaged 1.0, and no J_{hc} could be demonstrated for glucose. We conclude that J_{hc} is a high-capacity, low-affinity amino acid flux which passes through an intracellular solute pool, and which is sensitive to Hg at both the brush border and the basolateral cell membrane. If calculation of the saturation constants of aspartate reabsorption is restricted to experiments in which U/P <1.0, i.e. where J_{hc} is unlikely to contribute greatly to reabsorption, values some 20% lower than those previously reported are obtained; the Hg inhibition still is apparently uncompetitive in nature.

At low concentrations of filtered amino acids their tubular reabsorption is catalyzed by a family of specific carriers in the brush border membranes of renal proximal tubule cells (1); in addition, the mediation of their extrusion across basolateral cell membranes also helps determine the characteristics of the transepithelial flux (2). At high amino acid concentrations, active transcellular transport of at least some amino acids (1) is accompanied in the microperfused rat tubule by a reabsorptive flux which does not reach saturation; this high-capacity, low-affinity flux will be referred to as J_{hc}. The work reported here was undertaken to confirm in the intact kidney the existence of J_{hc}, to explore its nature, and to evaluate its impact on previously calculated values of the kinetic constants of amino acid reabsorption in control and Hg-treated animals (3).

Methods and Materials. As in earlier work, the animals used for the results reported here were New Zealand white rabbits (males, average body weight 2.5 kg). Mercury (10 μmole $HgCl_2$/kg) when required was injected sc 2 days previously; this treatment reduced the control single kidney GFR from 4.9 ± 1.2 ml/min (SD, $n = 17$) to 1.5 ± 0.6 ($n = 13$) ml/min. The animals were studied under nembutal anesthesia and killed at the end of the experiment before recovery from anesthesia. Clearance studies were carried out either with conventional iv sustaining infusions, or with the arterial gradient infusion technique previously described (3). With this procedure the increased recirculation of an infused solute is continuously counterbalanced by an exponential decrease of its concentration in the infusate. As a result, near step-wise increases in arterial solute concentrations can be achieved and maintained for several minutes. Under those conditions, over 80% of ^{14}C infused as aspartate remains in its original form in the renal artery (3). Moreover, because excreted ^{14}C following infusion of [^{14}C]aspartate as described here never enters the tubule cells (4), no significant metabolic breakdown of excreted [^{14}C]aspartate is expected. ^{14}C can therefore be used as tracer for aspartate under the present conditions. Fractional excretion of filtered solute was calculated using [^{3}H]methoxyinulin (sp act 358 mCi/g, ICN Radiochemicals) as glomerular marker, and dividing the ratio $^{14}C/^{3}H$ in urine by that in arterial plasma (steady-state experiments) or in bolus diluted with whole blood (transient experiments). The transepithelial transit time (TET) of cycloleucine was equated as previously to the ratio of tissue solute pool/

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reabsorptive flux (S/J) under steady state conditions (see Ref. two; note the more correct use of the symbol J for flux instead of the previously used K). Because of the relatively low fractional reabsorption (FR) values encountered in this work, it was necessary to subtract ^{14}C trapped in the lumen from the total tissue counts after these had been corrected as usual for counts in a 25% extracellular volume (2). As tissue inulin after correction for extracellular inulin may be equated to that trapped in the tubular urine, luminal ^{14}C could be calculated as the product of tissue inulin and fractional ^{14}C excretion. Creatinine was estimated in plasma and urine by the technique of Bonsnes and Taussky (5). All urine samples were collected through ureteral catheters. Radioactivity was counted in dpm on a Packard Model 2000 CA scintillation spectrometer. Plasma concentrations of administered amino acids were obtained from their specific activity in the infusate and the radioactivity of the plasma; low endogenous values of aspartate were neglected. The radioactive amino acids ([3-^{14}C]NH_2 isobutyric acid, AIB, 19.9 mCi/mmole, [*carboxy*-^{14}C]cycloleucine, 48 mCi/mmole, [U-^{14}C]aspartic acid, 220 mCi/mmole) were purchased from the New England Nuclear Corp. (Boston, MA).

Results. *Saturability of amino acid reabsorption.* Fractional reabsorption of filtered aspartate following arterial injection of boluses containing high concentrations of this amino acid did not approach zero as expected from a fully saturable process; instead, it plateaued at somewhat above 50% (results not shown). Because the bolus technique is incapable of relating results to a specific plasma concentration of the injected solute, the experiments were repeated under steady-state conditions. For the metabolizable amino acid aspartate, the steady state was achieved by arterial gradient infusion (see Materials and Methods). As shown in Fig. 1, the ratio of aspartate clearance/GFR in animals did not rise above approximately 50%; similar results were obtained with AIB. Interpretation of the clearance ratio of cycloleucine (cyclo) is less clear cut: Its fractional excretion rises much more slowly with plasma concentration than does that of aspartate (ASP). This makes it difficult to establish a clear plateau. However, even at the highest tolerated plasma concentrations, the fractional excretion of cycloleucine was only about 50%, as in the case of the two other amino acids. In each case, a high-capacity low-affinity reabsorptive flux (J_{hc}) appears to operate at high plasma levels.

Characteristics of J_{hc}. To characterize J_{hc}, attempts were made to measure the time required for the flux to cross the tubular epithelium (TET). Direct measurements with the rapid transient procedure (2) did not yield consistent results; a plausible explanation for this fact, as suggested under Discussion, lies in the limiting rate of solute equilibration between plasma and red cells. TET was therefore estimated indirectly from the equation TET = S/J in steady-state experiments. Results are shown in Fig. 2. Calculated values of TET are here plotted against the total reabsorptive flux (J, in μmole/min); J was normalized for GFR to permit comparison of control with Hg-treated animals. At low plasma levels in control animals, TET of cycloleucine is 0.7 min (2). As plasma levels were raised to the maximally achievable values, J/GFR rose to above 8 μmole/ml, while TET approximated 2–3 min. In Hg-treated animals at low cycloleucine concentrations, inhibition of basolateral extrusion of the acid prolongs TET from the control value of 0.7 min to over 10 min (6). As the concentration of cycloleucine was raised to achieve maximal values of J/GFR, great variability was observed in TET. However, the values remained uniformly higher than those in control animals, indicating that J_{hc} is sensitive to delay by Hg. These results do not confirm a preliminary report on the fall of TET with increasing J/GFR values in Hg-poisoned rabbits (7).

Figure 3 further shows the sensitivity of J_{hc} for cycloleucine and aspartate to Hg. At maximal concentrations of these amino acids their fractional reabsorption was significantly reduced in Hg-injected in contrast to control animals.

Influence of J_{hc} on calculated values of T_M and K_M. The occurrence of J_{hc} had not been taken into account in previous calculations of maximal tubular aspartate reabsorption (T_M) and the apparent affinity constant of the system, K_M (3). These calculations were

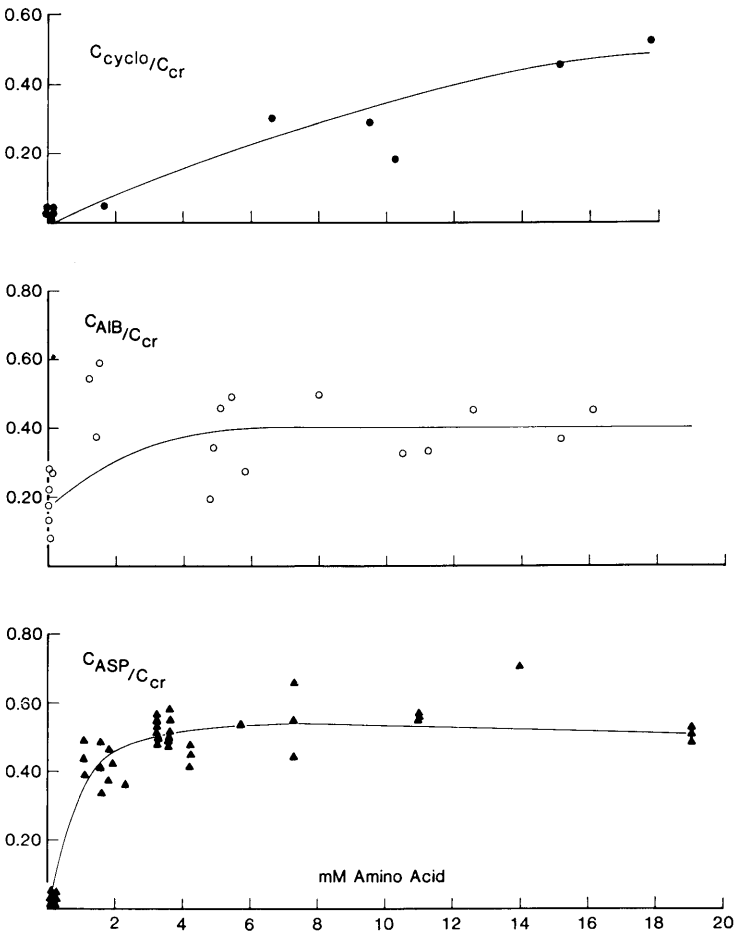

FIG. 1. Amino acid clearance ratios at varying plasma concentrations. All animals received a constant iv infusion containing 50 mg mannitol and 1 mg creatinine/ml saline at 1 ml/kg/min. For ASP, each point represents the mean from two successive 1-min urine samples, collected jointly from both kidneys in one animal; the urine collections were begun 1.5 min after initiation of arterial gradient infusion, and arterial blood was obtained after 1.5 and 2.5 min. For cycloleucine (cyclo) and AIB the values represent means of four 2-min clearance periods (two successive periods from each kidney), begun 20 min or longer after initiation of a constant iv infusion; arterial blood was collected at the end of the first of each pair of clearance periods.

based on 18 clearance values obtained in six rabbits, and each value represented the mean of two consecutive clearance periods, averaged for both kidneys. The T_M calculated from these means was 4.7 μmole/ml filtrate, and the K_M equaled 4.7 mM. If these values are recalculated with the exclusion of four periods in which U/P of aspartate exceeded 1.0, the remaining periods yield values for T_M for the high-affinity low-capacity transport system of 3.4 μmole/ml filtrate, and 3.2 mM for K_M.

When clearance periods for Hg-treated rabbits were similarly excluded from earlier results whenever (U/P) of aspartate exceeded 1.0, insufficient information remained to justify calculation of the Hg effects on the saturation constants. Additional studies were therefore performed with Hg at lower amino acid concentrations, under conditions where U/P remained below 1.0 despite the depression of aspartate reabsorption; results are shown in Fig. 4. The total of 12 clearance periods yields values of T_M of 1.0 μmole/ml

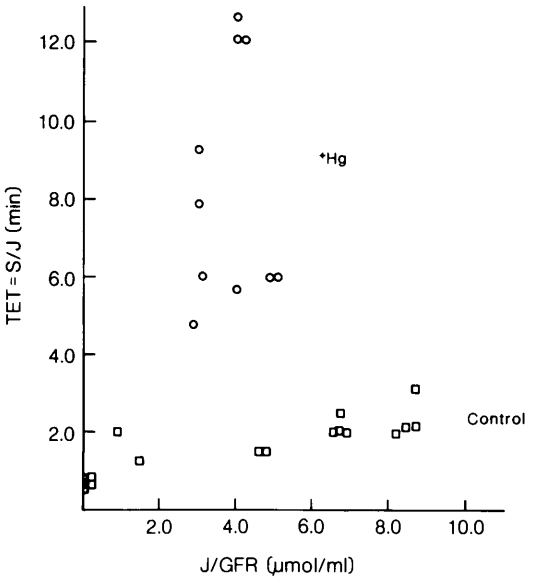

FIG. 2. TET for cycloleucine as function of reabsorptive flux in normal and Hg-treated rabbits. The control animals (□) were the same as those shown in Fig. 1; the Hg animals (○) had received sc 10 μmole Hg as $HgCl_2$/kg 2 days previously. Each point represents result from one kidney. TET was calculated indirectly as the ratio of *S/J*.

filtrate, and K_M = 1.1 m*M*, both slightly lower than previously reported. However, the earlier conclusion that Hg lowers both constants is obviously fully supported.

Specificity of J_{hc}. Whereas a high-capacity reabsorptive flux could readily be demonstrated for amino acids, no passive back leak of creatinine was observed: in 24 successive Hg-injected animals the clearance ratio of creatinine to inulin equaled 0.89 ± 0.11 (SD), a value identical to that seen in control animals and not significantly different from 1.0. We could also confirm in four rabbits under the present experimental conditions that tubular reabsorption of unlabeled glucose reaches a steady T_M value at high plasma levels (results not shown); no evidence was found for a nonsaturable reabsorptive flux.

Discussion. As reviewed by Silbernagl *et al.* (1), filtered amino acids at low concentrations are actively and essentially completely reabsorbed in early portions of the proximal tubule; at higher concentrations an apparently nonsaturable proximal component of reabsorption becomes evident, at least for some amino acids. The evidence presented here confirms and extends the finding of this high-capacity low-affinity component J_{hc} of amino acid reabsorption: above a steady plasma concentration of aspartate of 3–4 m*M* in clearance studies, or following injection of arterial boluses containing more than 100 μmole aspartate, the fractional excretion of filtered aspartate was observed to become

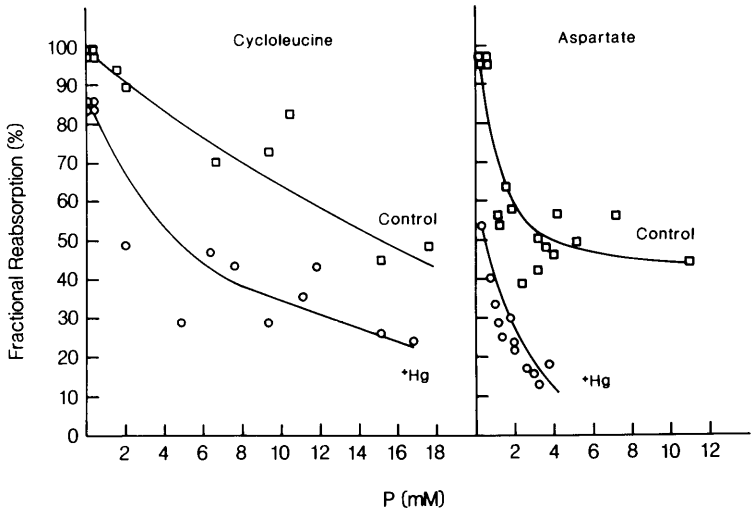

FIG. 3. Effect of Hg on J_{hc} of aspartate and cycloleucine. Fractional reabsorption (FR) was determined at different plasma concentrations (P) for cycloleucine following a constant iv infusion; aspartate was administered by arterial gradient infusion. Each point represents the mean of four 2-min clearance periods (two/kidney) from one animal. Curves were drawn by eye.

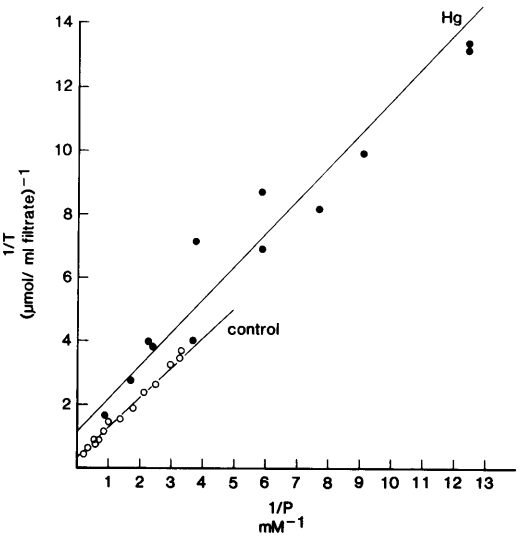

FIG. 4. Lineweaver–Burk plot of ASP reabsorption in control and Hg-treated animals. Each point represents the mean of two consecutive 1-min clearance periods, with values for each period averaged for the two kidneys. All U/P ratios <1.0. The 14 control values were taken from a previous publication (3); results from Hg-treated animals include four from the previous publication and an additional eight obtained in the present work.

independent of filtered load; similar results were obtained with AIB. Fractional reabsorption of cycloleucine also may plateau at around 50%, but this conclusion is less clear than for the other amino acids because of the high K_M of cycloleucine reabsorption (3).

At the high plasma concentrations where J_{hc} contributes significantly to total reabsorption the final urine/plasma (U/P) concentration ratio of the amino acids studied exceeded 1.0. These observations were made under conditions of strong mannitol diuresis and we may therefore assume that the proximal U/P ratio also exceeded unity. Similarly, following bolus injection of high concentrations of amino acids, the more rapid flow of peritubular blood than of tubular urine may cause their U/P ratio to exceed 1.0 after passage of the vascular concentration peak. The high capacity flux therefore moves amino acids down their concentration gradient.

Several arguments suggest that J_{hc} cannot follow paracellular diffusion pathways. In the first place, it is difficult to conceive of a membrane with leaky junctions permitting passive transfer of amino acids, but not leading to significant reabsorption of filtered creatinine or glucose. Secondly, the observation that S/J become relatively independent of J at high filtered loads (Fig. 2) indicates that the size of the pool becomes a function of J, so that J presumably passes through S; if J_{hc} were a paracellular event, S would have to accumulate in the intercellular spaces. A third argument, while not by itself excluding paracellular pathways, also supports the postulated transcellular J_{hc}. This is based on the action of Hg, shown in Fig. 3 to inhibit reabsorption both at low and at high amino acid concentrations. While Hg inhibition of paracellular solute flux could result from physical factors such as swelling of cells, it is difficult to see how such an effect could lead to the doubling of an extracellular solute pool in the tissue, as would be implied by the increased S seen in Figure 2. Instead it may be suggested that at high as at low amino acid concentrations Hg inhibits both influx and extrusion from an intracellular pool. Reduced fractional reabsorption reflects an effect of the metal at the brush border (8), while the increase in S results from depression of basolateral extrusion (6). Similar high-capacity low-affinity Hg-sensitive mechanisms therefore appear to operate on both sides of the cells.

An alternative explanation for our results was suggested by a reviewer. It postulates that the early proximal tubule possesses a high-capacity system permitting passive leak of amino acids along paracellular pathways; as the amino acid concentration is increased, more amino acid can thus be passively absorbed but more would also be delivered to the proximal straight tubule. If the paracellular permeability in the straight segment is much lower than in the early segments, active transport in the straight segment would increase cellular accumulation as filtered load of the amino acid increases. The net result of these processes would indeed be an increase in both S and J_{hc}. This explanation is based on the implicit assumption that the K_M of active amino acid reabsorption in the straight segment is very much higher than that in the early proximal tubule. With rising plasma concentrations the early proximal section would appear to have become saturated at around 3 mM (see Fig. 1); fractional reabsorption due to active transport in $S3$,

however, would have to remain constant up to a plasma concentration of at least 18 m*M*. This does not seem likely. Further, we are not aware of evidence that the passive permeability of *S*3 is much smaller than that of earlier segments. Finally, this alternative explanation does not escape the difficulty posed by the absence of creatinine or glucose leak out of the tubular urine.

Attempts to confirm the indirectly calculated values of TET at very high plasma concentrations by direct measurement following arterial bolus injection (2) yielded inconsistent results. One of the difficulties at very high transient plasma concentrations of amino acids may be that their equilibration between plasma and erythrocytes is relatively slow. Following bolus injection, the amino acid/inulin ratio in glomerular filtrate may therefore significantly exceed that subsequently measured in arterial samples. Another problem is posed by the limiting solubility of cycloleucine in the bolus.

As mentioned, our earlier study of the effects of metals on the kinetic constants of amino acid reabsorption (3) had not taken into account the occurrence of J_{hc}. A repeated analysis of these findings, excluding all clearance periods in which U/P exceeded 1.0, and adding results of additional experiments, fully confirmed the earlier conclusion that the heavy metal depresses both T_M and K_M, i.e., that it inhibits active amino acid reabsorption in an apparently uncompetitive manner. The toxicological significance of this observation remains in question (6).

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