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Na^+-dependent *p*-aminohippurate transport at the basolateral side of the isolated perfused rat kidney

Noriko Itoh, Yasufumi Sawada, Yuichi Sugiyama, Tatsuji Iga * and Manabu Hanano

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113 (Japan)

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The uptake of *p*-amino[^{3}H]hippurate by isolated perfused rat kidney was studied to characterize the mechanism which was responsible for organic anion transport process. A rapid injection multiple indicator dilution technique and the distributed two-compartment model of Sawada et al. (Computer Methods Programs Biomed., 20 (1985) 51) were employed. Some characteristics of a carrier-mediated transport from the antiluminal space to the intracellular space for *p*-aminohippurate at the basolateral side were demonstrated: the uptake was stimulated by the countertransport effect and showed Na^+ dependency. These findings are consistent with *p*-amino[^{3}H]hippurate's being taken up into the isolated rat basolateral membrane vesicle by Na^+-dependent carrier-mediated transport (J. Pharmacol. Exp. Ther. 227 (1983) 122). It is suggested that the multiple indicator dilution technique is a sensitive new method to study the mechanisms of renal tubular transport in the living kidney as an organ.

Introduction

Secretion of an organic anion, *p*-aminohippurate, is a carrier-mediated, active transport process, localized in the proximal tubule of the mammalian kidney [1]. *p*-Aminohippurate is concentrated intracellularly via an active transport step across the basolateral membrane [2] and subsequently passes into the urine by facilitated diffusion [3]. There are many reports dealing with tubular transport of various substances based on in vitro studies, using isolated membrane vesicles [4,5], isolated renal tubules [6] and renal cortical slices [7]. Kasher et al. [5] examined the relationship between the transmembrane Na^+-gradient and *p*-aminohippurate transport in isolated rat basolateral membrane vesicles. The Na^+-gradient (out → in) accelated the influx of *p*-amino[^{3}H]hippurate, whereas similar gradients of choline$^+$, K^+ or Li^+ did not. Sheikh and Moller [7] examined the transport of *p*-aminohippurate by the use of a preparation of rabbit kidney basolateral membrane vesicles and in rat kidney cortex slices under anaerobic conditions and obtained clear evidence of Na^+-gradient stimulation of *p*-aminohippurate transport with both preparations. Datzler and Bentley [6] studied effects of low Na^+ concentration on *p*-aminohippurate transport by isolated perfused snake distal-proximal renal tubules. Replacement of Na^+ in the bath by choline$^+$ led to significant depression of the net transport of *p*-aminohippurate from the bath to the lumen. However, there is no report concerned with the effect of Na^+ on the *p*-aminohippurate uptake using isolated perfused whole kidney system, which is akin to in vivo kidney. In order to estimate the intrinsic unidirectional transport parameter of

* To whom all correspondence should be addressed.

substances from the antiluminal (basolateral) side to the intracellular side in isolated perfused kidney, it is necessary to develop pharmacokinetic and tracer kinetic models based on experimental findings on the microcirculation of postglomerular site in the kidney.

In this study, we have developed the multiple indicator dilution technique for the measurement of tubular secretory parameters of *p*-aminohippurate in the isolated perfused rat kidney and determined the unidirectional rate constant (from the basolateral side to the intracellular side). Furthermore, we studied the counter-transport effect of the *p*-aminohippurate uptake at the basolateral membrane, and the effect of Na^+ replacement on the *p*-aminohippurate uptake from the antiluminal side to the intracellular side.

Methods

Kidney isolation and perfusion

Male Wistar rats weighing 380–430 g were anesthetized with diethyl ether, and the right kidney was operated according to the method described by Nishitsutsuji-Uwo et al. [8]. The artery, the renal vein and the ureter were cannulated. An 18-gauge needle connected to silicon tubing (internal diameter 2 mm, external diameter 3 mm) for the venous cannula, and PE 10 polyethylene tubing (internal diameter 2 mm, and external diameter 3 mm) for the venous cannula, and PE 10 polyethylene tubing (internal diameter 0.58 mm, external diameter 0.61 mm) for the ureteral cannula. Immediately after the operation, perfusion was started using a constant infusion pump (Mitsumi Science Co. Ltd., Tokyo, Japan), then the right kidney was isolated and connected to the perfusion apparatus. The kidney was placed on the apparatus, which was maintained at 37°C, and the perfusion pressure was kept at 100 ± 10 mmHg during the experimental period. Krebs-Henseleit buffer (118 mM NaCl/4.7 mM KCl/2.5 mM $CaCl_2$/1.19 mM KH_2PO_4/1.19 mM $MgSO_4 \cdot 7H_2O$/25 mM $NaHCO_3$) containing 6% bovine serum albumin, 10 mM glucose, 0.02% creatinine and 0.1% mannitol oxygenated with 95% O_2/5% CO_2 to pH 7.4 was used as the perfusate.

Renal function of isolated perfused kidney

The urine was sampled from the ureter at 10 min intervals via PE 10 polyethylene tubing. The glucose reabsorption ratio was measured according to the usual method. The the glucose reabsorption ratio was measured according to the method described by Whiteside and Silverman [9].

Light microscopy of kidney specimens

After perfusion experiments, the kidney was washed with distilled water. The tissue fragments were fixed in 4% neutral buffered formaldehyde solution, and paraplast sections were stained conventionally with hematoxylin and eoxin.

Multiple Indicator Dilution Method

The pulse-injection multiple indicator dilution technique developed by Goresky [10] was used. The injection solution (0.2 ml) for the study of *p*-aminohippurate transport consisted of T1824-labeled albumin (0.7 mg T1824 and 12 mg bovine serum albumin), 1.7 μCi of *p*-amino[^{3}H]hippurate and 0.17 μCi of [^{14}C]creatinine (the extracellular reference). In order to carry out the countertransport study, unlabelled *p*-aminohippurate or tetraethylammonium bromide was added to the perfusate (reservoir (R) in system 1 of Fig. 1). The final *p*-aminohippurate or tetraethylammonium bromide concentration in this perfusate was 1 mM. After cannulation procedure the right kidney was transferred into the perfusate cabinet. Perfusion with drug-free buffer medium in the system 2 was initiated immediately by connecting the renal arterial cannula to the outflow part (No. 4, Scheme III in Fig. 1). After 10 min, the injection solution was administered by a pulse-injection through the silicon tube cannula to the right renal artery. This is a control experiment for the countertransport study. After 5 min perfusion, the renal arterial cannula was disconnected from the system 2 and was quickly connected to the system 1 containing the reservoir (R) of *p*-aminohippurate or tetraethylammonium bromide buffer (no. 2, Scheme II in Fig. 1). After 10 min perfusion to obtain the steady state of *p*-aminohippurate level, the renal arterial cannula was disconnected from the system 1 and was quickly connected to the system 2 containing reservoir (R) of *p*-aminohippurate or tetraethylammonium bromide-free buffer (No. 4, Schema III in Fig. 1). After 4 s, the injection solution was administered by a pulse-injection

through the silicone tube cannula to the right renal artery. This is the *p*-aminohippurate or tetraethylammonium bromide preloading study for the countertransport study).

In order to examine the Na^+ dependency on *p*-amino[^{3}H]hippurate uptake, the regular perfusion medium (Krebs-Henseleit buffer) containing NaCl and $NaHCO_3$ in both the reservoirs (R) of system 1 and system 2, were replaced by LiCl and Li_2CO_3, respectively. The right kidney was transferred in the perfusion cabinet and the perfusion with Li^+ containing buffer medium in the system 2 was initiated immediately by connecting the renal arterial cannula to the outflow part (No. 4, Scheme III in Fig. 1). After 10 min, the multiple indicator dilution study (the first time) was carried out in the same way as the countertransport study. After 5 min perfusion, the renal arterial cannula was disconnected from the system 2 and was quickly connected to the system 1 containing Na^+-buffer (No. 2, Scheme II in Fig. 1). After 10 min perfusion, the multiple indicator dilution study (the second time) was carried out.

In all the study, the usual collections consisted of 20 perfusate samples collected at the rate of one sample per second. At the end of each experiment, the right kidney was excised, weighed and examined for any gross abnormality. The mean transit time for each material was calculated based on the outflow fraction over a period of 20 s by using the following equation:

$$\bar{t} = \sum_{t=0}^{20} C(t)t / \sum_{t=0}^{20} C(t) \qquad (1)$$

where t and $C(t)$ are the time and the concentration of the material at time t, respectively. The mean transit time was corrected for the catheter delay by subtracting the catheter mean transit time (0.82 s) calculated from the ratio of the catheter volume to the perfusion rate. The delay became a part of the large vessel transit time (t_0).

Assay

Radioactivities of *p*-amino[^{3}H]hippurate and [^{14}H]creatinine in the sample solution were determined in a Tri-Carb liquid scintillation spectrometer (model 3255, Packard Instruments Corp., Downers Grove, IL). 50 μl perfusate sample was added to a scintillation vial containing 10 ml of scintillation cocktail (0.1 g POPO, 4.0 g PPO and 500 ml Triton X-100/liter of toluene). An appropriate crossover correction was given to separate the two radioactivities of ^{3}H and ^{14}C. For T1824-labeled albumin, 50 μl of perfusate was diluted with 3 ml of distilled water and the concentration of T1824 was immediately measured at 610 nm in a double-wavelength double-beam spectrophotometer (Hitachi 557, Hitachi Co. Ltd., Tokyo, Japan). The concentrations of creatinine and glucose were determined using commercial kits (Creatinine-Test Wako and Glucose-B-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively) at 505 nm for creatinine and 520 nm for glucose in a double-wavelength double-beam spectrophotometer (Hitachi 557).

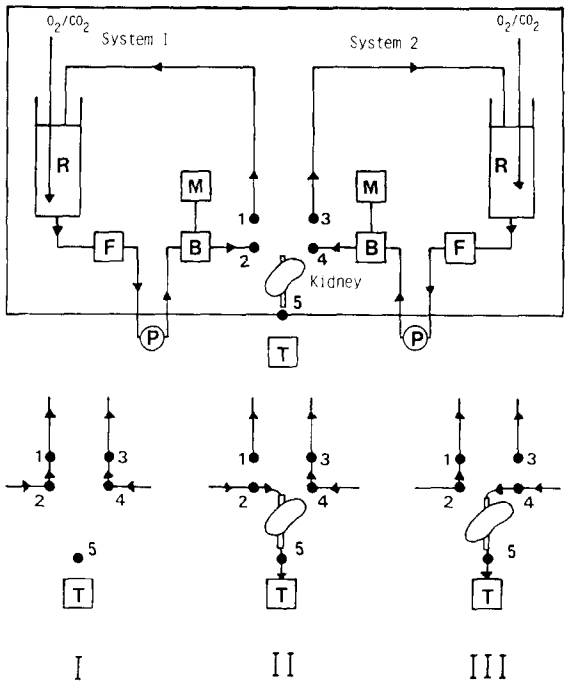

Fig. 1. Schematic representation of the rat kidney perfusion system. The renal arterial and caval connections of the kidney to the individual perfusion circuits are shown for each phase of the experiment: warm-up (I), perfusion for system 1 (II) and perfusion for system 2 (III). Key: P, pump; F, filter; B, bubble trap; M, manometer; and T, turntable.

Materials

p-Amino[*glycyl*-2-^{3}H]hippuric acid (1.8 Ci/mmol) and [*carbonyl*-^{14}C]creatinine hydrochloride (80 μCi/mg) were purchased from Amersham, Inc. (Arlington Heights, IL). [G-^{3}H]Inulin (217 mCi/g) was purchased from New England Nuclear Corporation (Boston, MA). Bovine serum albumin (Fraction V) was purchased from Sigma Chemical Co. (St. Louis, MO). *p*-Aminohippuric acid sodium salt was purchased from Nakarai Chemicals Ltd. (Kyoto, Japan). All other chemicals were commercial products and of analytical grade.

Analysis

Analysis of indicator dilution curves. Data were analyzed according to the flow-limited diffusion model of Goresky et al. [11–4] and Sawada et al. [15]. The concentration of *p*-aminohippurate in the effluent at time t is expressed by

$$C_{p\text{-ah}}(t) = \exp\{(-K_1 \cdot t) \cdot C_{cre}(t) + \exp-(K_2 + K_3) \cdot t\} \times \int_0^t \exp\{-(K_1 - K_2 - K_3) \cdot \tau\} \times C_{cre}(\tau) \times \sqrt{\frac{K_1 \cdot K_2 \cdot \tau}{t - \tau}} \times I_1 \cdot [2 \cdot K_1 \cdot K_2 \cdot \tau \cdot (t - \tau)]\, d\tau \quad (2)$$

and

$$K_1 = k_1\theta/(1+\gamma) \quad (3)$$

where γ is the ratio of the extracellular space to the plasma space, θ is the ratio of the cellular space to the plasma space, τ is the capillary transit time (dummy variable), $C_{cre}(t)$ is the concentration of creatinine as the extracellular reference appearing at the outflow, $C_{p\text{-ah}}(t)$ is the concentration of *p*-aminohippurate appearing at the outflow, K_1 is the influx rate constant from the extracellular space to the cellular space, K_2 is the efflux rate constant from the cellular space to the extracellular space and K_3 is the sequestration rate constant from the cellular space to the luminal side.

Experimental data were fitted to Eqn. 2 to obtain the values of K_1, K_2 and K_3 by a nonlinear least-squares method using digital computer as described previously [15,16]. The coefficient of variation of the fit was quantitated as

$$\text{C.V.} = \frac{\sqrt{\sum_{i=1}^{n} (\log \exp_i - \log \text{calc}_i)^2/(n-3)}}{(1/n)\sum_{i=1}^{n} \log \exp_i} \quad (4)$$

where n is the total number of data points, $\exp_i$ is the experimentally observed value at point i, and calc_i is the corresponding calculated value. At the early phase after the bolus injection, Eqn. 2 may be transformed into

$$C_{p\text{-ah}}(t) = \exp(-K_1 \cdot t) C_{cre}(t) \quad (5)$$

so that

$$\ln[C_{cre}(t)/C_{p\text{-ah}}(t)] = K_1 \cdot t \quad (6)$$

A semilogarithmic plot of the ratio of [^{14}C]-creatinine to *p*-amino[^{3}H]hippurate versus time generated a straight line with positive slope over the initial second following the bolus injection (Fig. 2). As defined by Goresky et al. [12,17], the magnitude of this slope is equivalent to $K_1 (= k_1\theta/(1+\gamma))$ (see Eqn. 3). In this paper, the relationship between the $\ln(C_{cre}/C_{p\text{-ah}})$ and time (t) is shown in Fig. 2. The apparent initial slope of a plot of the natural logarithm of the ratio of the ([^{14}C]creatinine) to *p*-amino[^{3}H]hippurate outflow fraction) was used as the initial estimate of K_1 for determining the rate constants using a nonlinear least-squares program [15,16].

The distribution volumes of albumin and creatinine were calculated by means of the following equations:

$$V_{d_{alb}} = F \cdot (\bar{t}_{alb} - \bar{t}_0) \quad (7)$$

$$V_{d_{cre}} = F \cdot (\bar{t}_{cre} - \bar{t}_0) \quad (8)$$

where F, $\bar{t}_{alb}$, $\bar{t}_{cre}$ and t_0 are the total flow through the system, the mean transit time of albumin, the mean transit time of creatinine and the large vessel transit time, respectively.

Statistical Analysis. All means are presented with their standard errors (the mean ± S.E.). Statistical significance was analyzed according to Student's *t*-test, using two tails.

Results

Renal function of the isolated kidney

All experiments were performed within 40 min after initiation of perfusion. During perfusion, isolated kidneys maintained a normal uniform color without mottling. Perfusion rates were 6–10 ml/min per g kidney with a perfusion pressure of 100 ± 10 mmHg. Urine flow was approx. 60–100 $\mu l \cdot min^{-1}$. During all experiments (40 min), GFR and the reabsorption ratio of glucose were 180–200 $\mu l \cdot min^{-1}$ and 90–99%, respectively. Replacement of Na^+ in the perfusate by Li^+ did not change the functional parameters except for the reabsorption ratio of glucose, which significantly ($p < 0.01$) decreased to 85–90%. As shown in Table I, the plasma volume and the extracellular volume were unchanged among various experiments.

Light microscopy of kidney specimens

Swelling of the tubular cells was not observed before and after perfusion.

Countertransport effect on p-amino[^{3}H]hippurate uptake at the basolateral side

Fig. 2 shows the relationship between ln ([^{14}C]creatinine/*p*-amino[^{3}H]hippurate) and time (t) (this is called the ratio plot). The ratio plots generated straight lines with positive slopes over the initial 5 s following the bolus injection, which correspond to the initial estimates of the influx rate constant (K_1). The preloading effect of unlabeled *p*-aminohippurate (1 mM) on *p*-amino[^{3}H]hippurate uptake is shown in Fig. 2a. The apparent influx rate constant increased in the presence of intracellular *p*-aminohippurate. On the other hand, *p*-amino[^{3}H]hippurate uptake showed no alteration, when unlabeled tetraethylammonium bromide (1 mM) was used instead of unlabeled *p*-aminohippurate (1 mM) (see Fig. 2b). The influx rate constant ($K_1 = k_1\theta/(1+\gamma)$) was calculated more precisely by the multiple indicator dilution-SALS method [15], which is an iterative non-linear least-squares method for analyzing the multiple indicator dilution data to obtain the kinetic parameters. The K_1 values for *p*-amino[^{3}H]hippurate in the presence (preloading) and absence of intracellular *p*-aminohippurate were 0.386 ± 0.042 s^{+1} and 0.322 ± 0.047 s^{-1} (mean ± S.E. of three experiments), respectively, and the difference was statistically significant ($p < 0.01$). However, no significant difference in the K_1 values for *p*-amino[^{3}H]hippurate was observed between the control (0.341 ± 0.033 s^{-1}) and the tetraethylammonium bromide preloading studies (0.280 ± 0.057 s^{-1}). Furthermore, neither K_2 nor K_3 values obtained simultaneously with K_1 value showed significant alteration in the *p*-aminohippurate- or tetraethylammonium bromide-preloaded kidney.

Effect of sodium replacement on p-amino[^{3}H]hippurate uptake at the basolateral side

Fig. 2c shows the effect of Na^+ replacement by Li^+ on the ratio plot ([^{14}C]creatinine outflow fraction)/(*p*-amino[^{3}H]hippurate outflow fraction)

TABLE I

EFFECT OF *p*-AMINOHIPPURATE, TETRAETHYLAMMONIUM BROMIDE AND Li^+ ON THE DISTRIBUTION VOLUME OF [T1824]ALBUMIN AND [^{14}C]CREATININE

See Analysis for details. Data represent the mean ± S.E. of three experiments.

	[T1824]Albumin (ml)	[^{14}C]Creatinine (ml)
p-Aminohippurate preloading		
control	0.53 ± 0.07	0.83 ± 0.08
+ *p*-aminohippurate	0.49 ± 0.07	0.91 ± 0.02
Tetraethylammonium bromide preloading		
control	0.52 ± 0.01	0.77 ± 0.02
+ tetraethylammonium bromide	0.45 ± 0.05	0.66 ± 0.05
Na^+ replacement		
control	0.73 ± 0.18	0.83 ± 0.06
+ Li^+	0.58 ± 0.09	0.80 ± 0.09

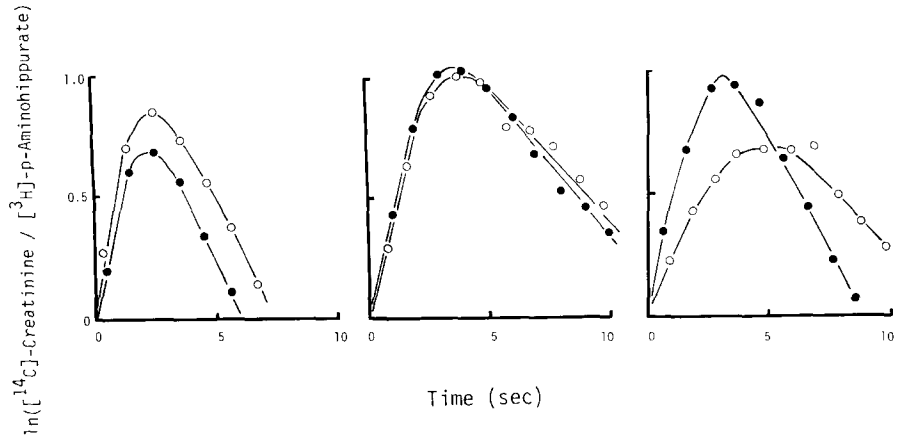

Fig. 2. Representative natural logarithm curves of the ratio (creatinine outflow fraction per milliliter/*p*-aminohippurate outflow fraction per milliliter) vs. time. Lines were calculated by the multiple indicator dilution-SALS method [15,16] using a digital computer. (a) Effect of unlabeled *p*-aminohippurate (1 mM) preloading on *p*-amino[^{3}H]hippurate uptake. Key: (●) control and (○) unlabeled *p*-aminohippurate (1 mM) preloaded. (b) Effect of unlabeled tetraethylammonium bromide (1 mM) preloading on *p*-amino[^{3}H]hippurate uptake. Key: (●) control and (○) unlabeled tetraethylammonium bromide preloaded (1 mM). (c) Effect of Na^+ replacement on *p*-amino[^{3}H]hippurate uptake. Key: (●) Li^+ buffer perfusion and (○) Na^+ buffer perfusion.

versus time). The apparent influx rate constant decreased in the Li^+ buffer perfused kidney. As the influx rate constant ($K_1 = k_1\theta/(1+\gamma)$ calculated by multiple indicator dilution-SALS method also significantly ($p < 0.01$) decreased from 0.435 (in the Na^+-buffer perfused kidney) to 0.308 ± 0.022 s^{-1} (in the Li^+-buffer perfused kidney; $n = 3$). However, neither K_2 nor K_3 values obtained simultaneously with K_1 value showed a significant alteration in the Li^+-buffer perfused kidney.

In order to show that the effect of Na^+ replacement by Li^+ was reversible, we changed the order of the use of Li^+ or Na^+ containing buffer. In one experiment, we perfused the kidney at first with Na^+ containing buffer, performed the multiple indicator dilution experiments for *p*-aminohippurate ($K_1 = 0.438$ s^{-1}, average of two flows), and after a 5 min stabilizing period, the perfusate was replaced by Li^+ containing buffer, and again performed the multiple indicator dilution experiments ($K_1 = 0.307$ s^{-1}, average of two flows). In the other experiment, we at first performed the multiple indicator dilution experiments in the Li^+-containing buffer ($K_1 = 0.309$ s^{-1}, average of two flows) followed by that in the Na^+-containing buffer ($K_1 = 0.397$ s^{-1}, average of two flows). These data indicated that the decrease in K_1 for *p*-aminohippurate induced by Na^+ replacement by Li^+ could be recovered by the addition of Na^+, suggesting that the effect of Li^+ on *p*-aminohippurate uptake was not due to the irreversible toxicity.

Discussion

Multiple indicator dilution study

To define the rate-limiting process for secreted substances at the renal tubule, it is important to determine the rates of influx (from the extracellular to the intracellular space), efflux (from the intracellular to the extracellular space) and sequestration (from the cell to the lumen). The multiple indicator dilution technique is useful for estimating separately these three parameters. This technique was originally applied to determine the vascular and extravascular volumes of the dog kidney in vivo by Chinard et al. [18–21]. Furthermore, Silverman et al. [22,23] investigated the chemical and steric requirements for monosaccharide interactions with the luminal and antiluminal surfaces of proximal tubule cells by the pulse-injection multiple indicator dilution technique. A recent study by Silverman et al. [24] attempted to examine the luminal and antiluminal uptake of glutamine in the normal and acutely or chronically acidotic dog by combining the pulse-

injection multiple indicator dilution technique with conventional arteriovenous difference measurements. However, in the organ such as the kidney, which has a heterogeneity of the capillary length, the pharmacokinetic analysis based on the distributed two-compartment model is necessary to estimate the tubular transport parameters from the multiple indicator dilution curves. Goresky et al. [25,26] have developed a pulse-injection multiple indicator dilution technique which made it possible to analyze the uptake process of materials by the liver, based on the distributed model. More recently, Silverman et al. [27,28] and Itoh et al. [29] investigated the permselectivity of the postglomerular capillary wall by utilizing the multiple indicator dilution technique in the dog kidney in vivo [27,28] and the isolated perfused rat kidney [29], using simultaneous injection of T1824-labeled albumin (the vascular reference) creatinine (the extracellular reference) and one or two radioactively labeled indicators. Furthermore, we developed a new method based on the multiple indicator dilution technique to study the kinetic relationships between the renal tubular cell uptake process and the secretory process of cimetidine [30].

In this study, we applied this method to the renal tubular transport study of *p*-aminohippurate and determined the influx rate constants ($K_1 = k_1\theta/(1+\gamma)$) from the antiluminal side to intracellular space. The coefficients of variation (see Analysis) as the criterion for the goodness-of-fit were ranged from 0.00453 to 0.0210. Itoh et al. [30] carried out the multiple indicator dilution studies on labeled cimetidine uptake using the isolated perfused rat kidney and reported the C.V. values (0.007–0.0100) which were comparable to those of the present study. Basic functions of the rat kidney used in this study was comparable with those of previous reports (see Results). No change was observed in the distribution volumes of the reference substances in the presence of 1 mM *p*-aminohippurate, 1 mM tetraethylammonium bromide or 143 mequiv. Li^+ as compared to the control (Table I). This means that the kidney viability was kept normal during the course of study in all experiments.

Countertransport of p-amino[^{3}H]hippurate at basolateral side

One of the common phenomena displayed by a membrane carrier transport system is the countertransport of labeled substrate [31]. We therefore designed an in situ experiment to search for this phenomena. As shown in Fig. 2a, the influx rate constant of tracer *p*-amino[^{3}H]hippurate increased in the presence of intracellular unlabeled *p*-aminohippurate. This transstimulation effect was not observed in the presence of intracellular unlabeled tetraethylammonium bromide (Fig. 2b). These findings suggest that the transport of *p*-aminohippurate at the basolateral side of the rat kidney tubule is due to the carrier-mediated transport process, which is different from the cation transport system and shows the transstimulation effect. Kinsella [3], Ross [5] and Hori [4] reported the same transstimulation effect as that shown in this study, using isolated rat or dog basolateral membrane vesicles.

Na^+ dependency of p-aminohippurate uptake

Evidence correlating *p*-aminohippurate transport with ($Na^+ + K^+$)-ATPase activity has been provided independently by several groups [32–37]. In these studies, ouabain or vanadate, which are known as inhibitors of the ($Na^+ + K^+$)-ATPase, produced a concentration-dependent decrease in the enzyme activity (as reflected by changes in the intracellular Na^+ and K^+ concentrations) and a corresponding decrease of *p*-aminohippurate uptake into renal slices. Recently, Kasher et al. [5] examined the relationship between the transmembrane Na^+-gradient and *p*-aminohippurate transport using isolated rat basolateral membrane vesicles. 100 mM Na^+ gradient (outside → inside) accelerated the influx of *p*-amino[^{3}H]hippurate, whereas a similar gradient of choline$^+$, K^+ or Li^+ did not. Sheikh et al. [7] studied *p*-aminohippurate transport using rabbit kidney basolateral membrane vesicles and rat kidney cortex slices. They obtained a clear evidence of Na^+-gradient stimulation of *p*-aminohippurate transport with both preparations. Dantzler et al. [6] studied the effect of the low Na^+ concentration on *p*-aminohippurate transport by isolated perfused snake distal-proximal renal tubules. Replacement by Na^+ in the bath with choline$^+$ produced a signifi-

cant depression of the net *p*-aminohippurate transport from the bath to the lumen.

As shown in Fig. 2c, when the kidney was perfused with Li^+-buffer, *p*-aminohippurate uptake was depressed on the average by 30%. Thus, it is unlikely that the decrease of *p*-aminohippurate uptake found in the absence of Na^+ results from the toxic damage by Li^+, since essential parameters such as GFR, blood flow, urine flow and the distribution volume of reference substances were virtually unchanged. From our present data, it is impossible to analyze the interaction of Na^+ with the *p*-aminohippurate transport system which Kasher et al. [5] stoichiometrically showed using isolated basolateral membrane vesicles. To our knowledge, however, this study is the first investigation in which isolated perfused rat kidney has been used to determine the effect of Na^+ on *p*-aminohippurate uptake.

In conclusion, we estimated the influx rate constant of *p*-aminohippurate from the antiluminal space to the intracellular space at the basolateral side and found out the transstimulation effect and Na^+-gradient stimulation effect on *p*-aminohippurate transport utilizing a multiple indicator dilution technique in the isolated perfused rat kidney.

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