Acid–Base Effects on Renal Organic Cation Transport[1] (37021)

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Mammalian renal tubules secrete strong organic cations such as tetraethylammonium (TEA), *N'*-methylnicotinamide and mepiperphenidol (Darstine) by a common route which is functionally distinct from the transport system for organic anions. Because of profound pharmacologic responses which may occur upon administration of some of these organic cations, many studies of this secretory transport pathway have been performed *in vitro* (2–4). While several parameters of this transport have been studied, little is known concerning the effects of acid–base balance upon it. The present study was conducted to determine how intracellular and extracellular factors in acidosis and alkalosis affect TEA transport in renal slices and to compare these observations with previous observations of hippurate transport (5).

Materials and Methods. Male Sprague-Dawley rats, 150–250 g, were used in all experiments. Rats were allowed free access to chow and drank water, NH_4Cl solution (1.0% w/v), or $NaHCO_3$ solution (1.5% w/v). The latter two solutions were drunk for a period of 7 to 10 days.

Rats were sacrificed by a blow to the head, the kidneys were rapidly removed and placed in cold saline. Cortical slices (0.4 to 0.5 mm) were cut with a Stadie-Riggs microtome (6) within the subsequent 30 min.

The basic incubation medium used was that described by Cross and Taggart (7) phosphate buffered sodium, potassium and chloride solution. To this, we added ^{14}C-tetraethylammonium bromide (TEA) (New England Nuclear) at a concentration of 10^{-5} *M*. In a few studies, ^{131}I–Na iodo hippurate at a concentration of 10^{-5} *M* was added. Medium pH was 7.4; however, in some studies, 0.1 *N* HCl or 0.1 *N* NaOH was added to the medium to obtain a range of pH. Following 90 min of incubation at 24° on a Dubnoff shaker, slices were removed, blotted and weighed. Tissue weights ranged between 30–50 mg. Slices were placed in 5% trichloroacetic acid, homogenized and then centrifuged. We used the supernatant for β counting. The medium in which incubation took place was also added to trichloroacetic acid, centrifuged and the supernatant was counted. The basic scintillation mixture was composed of triton X-100 (Packard Nuclear) (1/3 vol), toluene (2/3 vol), 2-5-diphenyloxazole (5.5 g/liter) and 1,4-bis[2-(5-phenyloxazolyl)] benzene (0.125 g/liter). β counting and quench correction were performed on a Packard counter, Model 2420. Gamma counting was performed with a γ well-type counter.

Results are expressed as S/M ratios; *i.e.*, the ratio of counts per minute per gram of tissue weight to the counts per minute per milliliter of incubation medium. Statistics are by Student's *t* test using paired or group analysis as noted in the text.

Results. Effects of medium pH changes. The ability of kidney slices to accumulate TEA in media at several pH levels was studied (Fig. 1). For comparison, hippurate transport accumulation from previous studies is shown. The highest TEA S/M ratio was found at pH 8.0 *i.e.*, S/M ratio of 9.7 ± 0.5 (SEM); the lowest at pH 5.5, S/M ratio of 5.4 ± 0.2 (SEM). At pH 6.5 to 8.5, a plateau for TEA accumulation (S/M 9.1 to 9.7) was seen. Although it appears that more accumu-

[1] Supported by NIH Grant AM 11525.

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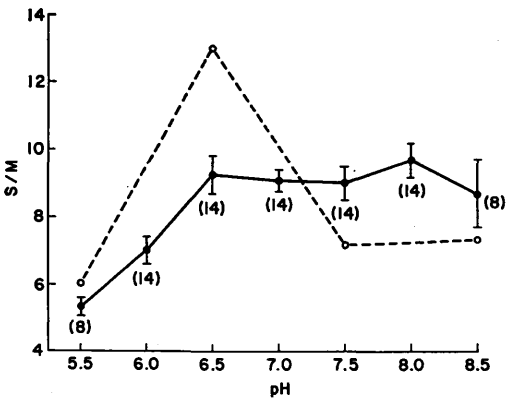

FIG. 1. S/M ratios for TEA transport at various medium pH. The SEM is depicted. Each point represents 8–14 determinations, performed on water drinking rats. (- -) The S/M for hippuric acid, which is shown for comparison.

lation occurs at 8.0, analysis of variance showed no significant difference at any pH above 6.5. As previously reported this differs from hippurate transport by rat kidney slices which showed a significantly higher S/M ratio at pH 6.5 (5).

Effects of sera from NH_4Cl and $NaHCO_3$ drinking rats. Sera were obtained from 26 rats drinking 1.5% $NaHCO_3$ solution and 26 rats drinking 1.0% NH_4Cl solution. Rat kidney slices from rats drinking plain water were halved so that one half of each slice could be tested in sera from the NH_4Cl or $NaHCO_3$ drinking rats. A serum from an NH_4Cl drinking rat was compared to a serum from a $NaHCO_3$ rat at a 10% (v/v) concentration of sera, media pH 7.4. In experiments depicted in Table I, sera from NH_4Cl drinking rats stimulated TEA accumulation when compared to sera obtained from $NaHCO_3$ drinking rats (↑ 12%). The TEA accumulation by slices just did prove to be statistically greater in sera from the NH_4Cl drinkers when using paired analysis ($T = 2.38$, $p < .05$). The S/M ratio was greater in the presence of the "acid sera" in 18 of 26 observations. When 15 sera from NH_4Cl drinking rats were compared with 15 sera from $NaHCO_3$ rats as to their ability to affect hippurate accumulation, the sera from the NH_4Cl drinking rats significantly stimulated hippurate accumulation (↑ 12%) as depicted in Table I and shown before (5).

TEA accumulation in kidney slices from chronically acidotic and alkalotic rats. In kidney slices from 33 rats drinking 1% NH_4Cl solution, the average TEA S/M ratio was 14.1 ± 0.8 (SEM) compared to a TEA S/M ratio of 16.9 ± 0.8 (SEM) in kidney slices from 33 $NaHCO_3$ drinking rats. Therefore, like hippurate transport (5), TEA transport was also greater in kidney slices from alkalotic rats when compared to acidotic rats ($T = 2.23$, $p < .05$). In a separate study, slices from 20 rats drinking water showed an average S/M of 14.7 ± 0.7 (SEM).

Discussion. The specificity and distinctness of the renal secretory pathways for naturally occurring and synthetic organic bases and organic anion transport has been established. It has been shown that accumulation of organic anions and cations occur simultaneously without mutual interference. Metabolic inhibitors have been noted to affect the tubular transport of each system differently. For example, fluoracetate and malonate depress PAH secretion *in vivo* but do not affect TEA transport. The optimal temperatures for PAH and organic cation transport differ; and as shown in the present study, the optimal pH for accumulation of these substances in rat kidney slices differ.

However, while many differences between the two transport systems exist, extra and intracellular events triggered by acidosis and alkalosis appear to have similar effects on the two transport systems. Using TEA as a representative organic cation, we found that slices from acidotic rats had decreased TEA transport and that sera from acidotic rats when compared to sera from alkalotic rats stimulated TEA transport.

Several hypotheses have to be examined to explain these results. Extracellular factors affecting transport systems may be related to changes in content of circulating metabolites. Low concentrations of normal sera can stimulate hippurate accumulation, while azotemic sera and high concentrations of normal sera depress accumulation (8–10). Based on the finding that these factors can be ultrafiltered and dialyzed, and are removed by cation exchange columns, we have postulated that some

TABLE I. Effects of Sera (10% v/v) from NH_4Cl Drinking (Acidotic) and $NaHCO_3$ Drinking (Alkalotic) Rats on TEA and Hippurate Transport in Kidney Slices.[a]

Sera	Observations	S/M	↑(%)	No. observations acid > alkaline
		^{14}C-TEA transport		
Acidotic	26	15.3 ± 1.0	12	18[b]
Alkalotic	26	13.7 ± 1.0		
		^{131}I–Na iodo hippurate transport		
Acidotic	15	14.6 ± 0.4	12	11[b]
Alkalotic	15	13.0 ± 0.5		

[a] Sera from 26 acidotic were paired with sera from 26 alkalotic rats and compared as to their ability to effect TEA transport in rat kidney slices, 10% (v/v) sera were used. With 15 sera from each group, hippurate transport in renal slices was also studied.

[b] $p < .05$.

of this effect may be due to stimulation by metabolizable cations such as acetate, lactate, *etc.*, (7, 9). Acid–base changes have been shown to affect levels of acetate, pyruvate and lactate (11–13) and may explain the relatively greater stimulatory effect of sera from acidotic slices on hippurate transport (5). Several of these compounds have been shown to modify TEA transport although perhaps to a lesser extent than PAH transport (1, 14). Changes in the concentration of these circulating organic anions during acidosis could theoretically affect organic base transport.

As for the intracellular effect, there is abundant evidence that metabolism of slices changes in response to acidosis. Slices from acidotic rats, in comparison to those from alkalotic rats, produce more ammonia and glucose (15) and consume more oxygen (16). Future studies may correlate these changes in metabolism with changes in various transport systems.

In summary, we can state that organic cation transport in slices is affected by the acid–base status of the rat, being higher in slices from alkalotic rats. Sera from acidotic rats stimulates, to a small extent, TEA transport when compared to sera from alkalotic rats. Over a wide range of pH (6.5–8.5), little effect on TEA transport is seen.

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Received May 26, 1972. P.S.E.B.M., 1973, Vol. 142.