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RELATIONSHIP BETWEEN 2,4,5-TRICHLOROPHENOXYACETATE AND THE RENAL ORGANIC BASE TRANSPORT SYSTEM*

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Abstract—At concentrations which did not alter tissue oxygen consumption, 2,4,5-trichlorophenoxyacetate (2,4,5-T) increased the rate of tetraethylammonium (TEA) efflux from renal slices by 35–130 per cent. The efflux of N^1-methylnicotinamide (NMN) was unaffected by 2,4,5-T. The steady-state transport of 2,4,5-T by renal slices was not altered by competitive or irreversible inhibitors of the base transport system. These data and others indicate that, despite 2,4,5-T–TEA interaction, 2,4,5-T is not transported totally or even to a great extent by the renal organic base secretory mechanism. A model is presented that is consistent with all the observations.

The uptake of tetraethylammonium (TEA) by renal cortical slices is decreased by competitors of the organic base transport system and by metabolic inhibitors [1, 2]. The slice accumulation of another organic cation, N^1-methylnicotinamide (NMN), is reduced by many of the same competitive or metabolic inhibitors [1, 2]. However, the efflux of NMN is not altered significantly by competitive inhibitors, but is reduced slightly by potent transport inhibitors [3] and significantly increased by dinitrophenol, an uncoupler of oxidative metabolism [4].

The steady-state accumulation and the initial rate of uptake of TEA are reduced in renal slices from 2,4,5-trichlorophenoxyacetate (2,4,5-T)-pretreated rats [5]. In addition, the efflux of TEA is increased in slices from pretreated animals [6]. The influence of 2,4,5-T on TEA accumulation in normal renal slices appears to be competitive in nature and occurs when the concentration of organic ions is at a high intracellular concentration [7]. Such a competitive interaction of TEA, an organic base, and 2,4,5-T, a compound transported by the organic anion system, is uncommon. That 2,4,5-T is transported by the *p*-aminohippurate (PAH) system is clear from earlier work with specific competitors [8, 9].

The present study was performed to determine the mechanism of 2,4,5-T alteration of TEA transport, i.e. whether 2,4,5-T had an inhibitory effect on oxygen consumption or acted as a classical competitive inhibitor. Also, the extent of 2,4,5-T–organic base interaction was studied with classical reversible and irreversible inhibitors of organic base transport.

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METHODS

The experimental procedures employed in this study were similar to those used in other studies from this laboratory [5–8]. Renal cortical slices were prepared from the kidneys of male Sprague–Dawley rats as reported earlier [8] and incubated in a modified Krebs–Ringer phosphate buffer at pH 7.4 [6, 10]. Slices were incubated at 25° in a Dubnoff metabolic shaker (100 cycles/min) with a 100% oxygen atmosphere. The renal slice transport of ^{14}C-labeled organic ions (Amersham/Searle or New England Nuclear) was measured using standard liquid scintillation techniques and an external standard for quench correction. The organic compounds studied were NMN, TEA, 2,4,5-T and PAH. At the end of the incubation period, the tissues were weighed and homogenized in distilled water. The ^{14}C concentration was determined in aliquots of the media or whole tissue homogenates. The uptake data are expressed as the slice-to-medium or S/M ratio (cpm/g of tissue ÷ cpm/ml of medium).

The procedure for determining the efflux of ^{14}C-labeled organic ions from renal cortical slices is a modification of that used by Farah *et al.* [11]. The slices were preloaded with the test compound for 60 min at 25° in the presence of 100 per cent oxygen. After this preincubation, the tissues were rinsed briefly and then transferred at 1-min intervals through a series of individual runout chambers each containing 3.0 ml of fresh Krebs–Ringer phosphate buffer at a constant temperature of 25°. Tissue transfer was accomplished by means of a nylon net attached to a rubber-coated metal wire. The radioactivity collected from each runout chamber after exposure of the tissue plus that remaining in the tissue after the experiment was used to construct the efflux curves and calculate the rate constants.

The oxygen consumption of renal slices was studied using a Yellow Springs Instruments model 53 oxygen monitor employing a Clark-type electrode. The data

B.P. 26/18—E

are presented as initial rates of oxygen consumption (μl O_2/hr/mg wet tissue) computed from the decrease in oxygen saturation of the 4-ml bathing solution over the first 5 min of a 10-min incubation. The standard Krebs–Ringer phosphate buffer solution was used as the bathing solution and was saturated with air prior to the start of incubation.

The data were analyzed statistically using the Student's t-test or a paired t-test depending on experimental design. The level of significance was chosen as $P < 0.05$.

RESULTS

Renal cortical slices from 2,4,5-T-pretreated animals have a depressed oxygen consumption when no metabolic substrate is present in the incubation medium during the measurement of oxygen uptake [6]. Such a disruption in cellular metabolism might explain the alterations found in TEA transport by renal slices [5, 6]. To determine whether 2,4,5-T can influence oxygen consumption of normal renal slices and thus influence organic cation transport, 10^{-3} M 2,4,5-T was added to the renal slice incubation medium. No significant influence on tissue respiration was observed whether or not lactate was present in the bathing solution (Fig. 1).

The runout of TEA from renal slices of rats pretreated with 2,4,5-T was significantly greater than from control slices [6]. A possible mechanism for the interaction between 2,4,5-T and TEA comes from direct competition studies which showed that 2,4,5-T competes for the steady-state transport of TEA [7]. To determine whether or not 2,4,5-T would increase TEA efflux from normal tissue preloaded with TEA, the efflux test procedure of Farah *et al.* [11] and Ross *et al.* [4] was used. TEA-preloaded tissues were transferred through a series of seven chambers containing herbicide-free buffer and the next eight chambers contained 10^{-4} or 10^{-3} M 2,4,5-T in the same buffer. The runout of TEA was measured and the first-order rate constants were calculated. Data from representative experiments are presented in Fig. 2 for TEA efflux and similar efflux experiments with tissues preloaded

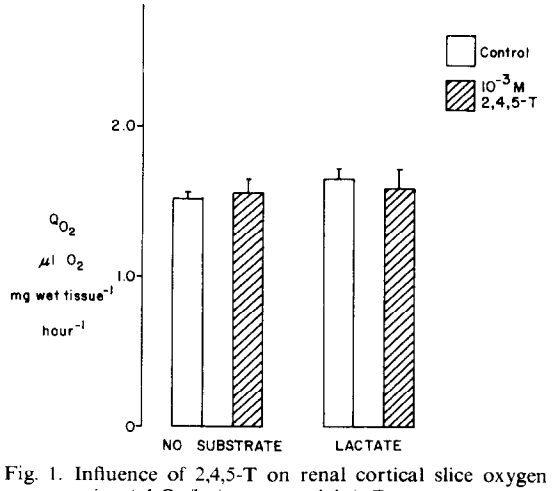

Fig. 1. Influence of 2,4,5-T on renal cortical slice oxygen consumption (μl O_2/hr/mg wet weight). Data are presented as mean values ± S.E. of five experiments. Lactate was present at a concentration of 10^{-2} M as indicated.

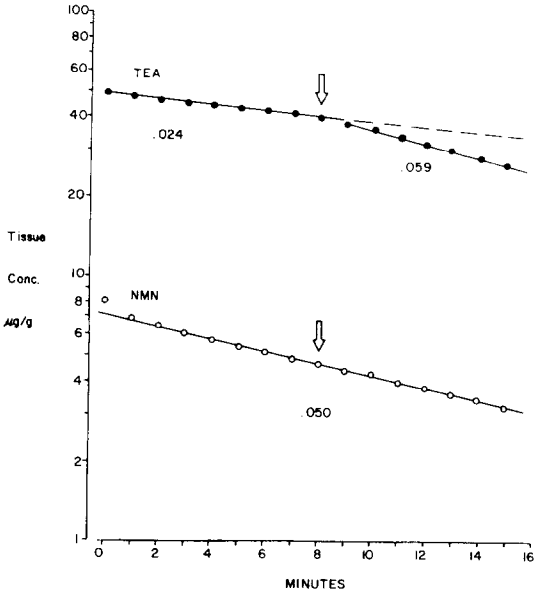

Fig. 2. Typical data for the effect of 2,4,5-T on the runout of TEA (●——●) and NMN (○——○). Arrows mark the addition of 10^{-3} M 2,4,5-T. The first-order rate constants are given for each line and are in units of reciprocal minutes.

with NMN. Although TEA efflux was increased within minutes of 2,4,5-T addition, no effect on NMN efflux was observed.

Summary data for the influence of 2,4,5-T on TEA and NMN efflux are presented in Fig. 3. The first-order rate constants for TEA determined from the runout in the presence of 2,4,5-T were significantly greater than the control values. The NMN runout rate constants were significantly less than those for TEA in the presence of 10^{-3} M 2,4,5-T (−22 per cent) and significantly greater than those for TEA control (+46 per cent).

Since certain metabolic and potent competitive inhibitors alter NMN efflux, these compounds were tested to determine their influence on TEA runout to compare with the influence of 2,4,5-T on TEA efflux. Although many studies on TEA transport have been performed, specific experiments on competition for runout were not done [12, 13]. As with NMN efflux, TEA runout was increased by dinitrophenol. Although a slight decrease in TEA runout was produced by mepiperphenidol, the effect was not significant (Table 1).

Since 2,4,5-T has been shown to inhibit TEA transport competitively, and TEA was ineffective in blocking 2,4,5-T transport [7], other potent inhibitors of the base transport system also were tested for their effects on 2,4,5-T transport. If these organic base transport inhibitors reduced 2,4,5-T transport, this would be evidence that 2,4,5-T not only alters TEA transport but also is transported by the base system. Cyanine dye No. 863, a competitive transport inhibitor, at a concentration (1.0 μg/ml) which markedly reduced TEA transport, failed to alter the transport of 2,4,5-T and PAH (Table 2). At higher concentrations of cyanine (2.5 to 5 μg/ml), 2,4,5-T transport was decreased, but so was that for PAH.

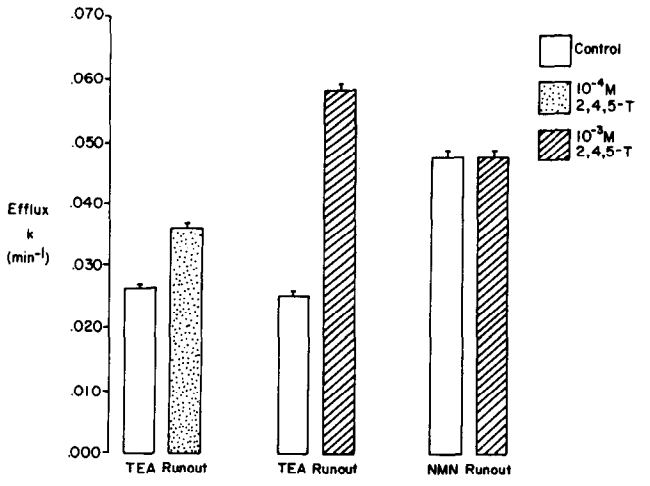

Fig. 3. Influence of 2,4,5-T on the first-order rate constants for the runout of organic bases. The data are presented as mean values ± S.E. (N = 4) in units of reciprocal minutes. Tissues were preloaded with 3.57×10^{-5} M TEA or 1.67×10^{-5} M NMN for 1 hr at 25°.

Table 1. Effect of inhibitors on TEA efflux*

	TEA efflux	
	Dinitrophenol	Mepiperphenidol
Control k, min^{-1}	0.0278 ± 0.002	0.0270 ± 0.001
Test k, min^{-1}	0.0542 ± 0.005†‡	0.0251 ± 0.002‡

* Data are presented as mean first-order rate constants ± S.E. in units of reciprocal minutes (N = 3). Tissues were preloaded with 3.57×10^{-5} M TEA for 1 hr at 25°. Inhibitors were present at a concentration of 10^{-4} M.
† $P < 0.05$.
‡ Dinitrophenol produced an increase of 95 per cent in test k compared to the control; mepiperphenidol produced a decrease of 7 per cent in test k compared to the control.

Phenoxybenzamine, an irreversible inhibitor of the organic base transport system [4], did not reduce 2,4,5-T transport significantly (Fig. 4). The lack of effect on 2,4,5-T was paralleled by a similar minor effect on PAH transport. Mepiperphenidol [14], an effective competitive inhibitor of the cation system, also had a minimal effect on 2,4,5-T transport (Fig. 4).

Table 2. Effect of cyanine dye No. 863 on TEA, 2,4,5-T and PAH accumulation*

	Control (S/M ratio ± S.E.)†	Cyanine No. 863 (S/M ratio ± S.E.)	Per cent change
TEA	8.20 ± 0.15	5.89 ± 0.18‡	−28.2
2,4,5-T	10.55 ± 0.30	10.73 ± 0.18	+1.7
PAH	12.38 ± 0.36	12.85 ± 0.24	+3.8

* Data are presented as mean values ± S.E. (N = 4). Renal cortical slices were incubated with organic ions (7.5×10^{-5} M) for 120 min. Cyanine dye No. 863 was present at a concentration of 1 μg/ml (mol. wt = 390).
† S/M = slice-to-medium.
‡ $P < 0.05$.

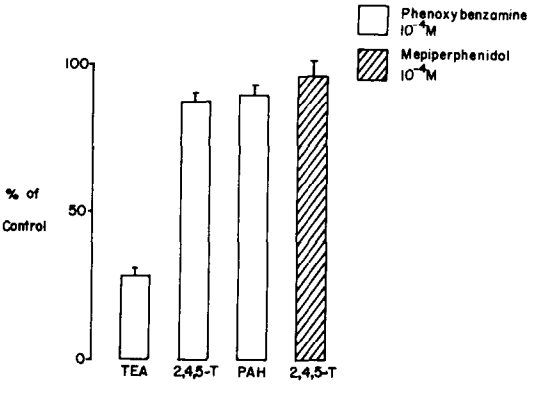

Fig. 4. Effect of phenoxybenzamine and mepiperphenidol on organic ion transport (N = 4). Data are presented as the per cent of control accumulation ± S.E. Renal slices were incubated with organic ions (7.5×10^{-5} M) for 120 min.

DISCUSSION

Previous studies have demonstrated that metabolic inhibitors decrease the uptake of TEA and NMN [1, 2]. The organic acid herbicide 2,4,5-T has been shown to reduce the transport of TEA readily, but has only minor effects on the renal slice transport of NMN [7]. These facts could be interpreted to mean 2,4,5-T does not alter TEA transport by means of disruption of cellular metabolism. The finding that 2,4,5-T (10^{-3} M) does not alter renal slice oxygen consumption supports this belief. However, it has been noted that 2,4,5-T can produce a decrease in oxygen consumption by renal tissue from animals pretreated with 2,4,5-T [6]. This decrease in oxygen consumption is reversible upon addition of transportable organic ions, which might relate to the reversal of the inhibited organic acid transport system, an energy-dependent transport process. That is, once a large concentration of organic ion was present and transported by the tissue, the inhibition was reversed [7] and normal metabolism resumed [6]. Such a transient reversal of oxygen consumption inhibition has

been reported in other animal cells and liver mitochondria [15].

The apparent competitive interaction of 2,4,5-T on intracellular TEA accumulation is supported by the current efflux studies. The large (30 to 130 per cent) and immediate (1–3 min) increase in efflux of TEA in the presence of 2,4,5-T is in agreement with a specific disruption of the intracellular accumulation process as seen by the increase in PAH efflux by organic acid competitive inhibitors. In the case of TEA, since classical base inhibitors fail to alter TEA efflux, a unique interaction of TEA and 2,4,5-T will be postulated (see below). The need for a reconsideration of these transport systems is emphasized by the lack of an effect of 2,4,5-T on NMN efflux. This observation is consistent with the lack of a significant effect of other competitive inhibitors on efflux [3].

Potent competitive (cyanine dye No. 863 and mepiperphenidol) and irreversible (phenoxybenzamine) inhibitors failed to alter 2,4,5-T transport. The lack of inhibition might by explained if 2,4,5-T is transported by both the organic anion [8, 9] and organic cation system. That is, because 2,4,5-T is accumulated by slices by both transport systems, when the base transport system is inhibited completely, 2,4,5-T accumulation could occur via the acid system and hence no depression of uptake would be observed. Attempts to test this hypothesis were undertaken by measuring the effects of organic base competitors on 2,4,5-T transport in the presence of high concentrations of probenecid. It was reasoned that the non-probenecid sensitive 2,4,5-T uptake might be blocked by base inhibitors thus revealing transport by this base system. These experiments yielded negative results and led to the alternate explanation presented in Fig. 5.

In this model it is proposed that 2,4,5-T does not interact with the carrier mediated aspect of base transport located in the plasma membrane, but rather at specific intracellular accumulation sites for TEA. This proposal is an adaptation of the two-step transport model for PAH secretion by the proximal tubule of mammals originally proposed by Copenhaver and Forster [16], and subsequently by Foulkes and Miller [17]. The base kinetic behavior of movement is virtually identical to that proposed for organic anions.

Two transport steps are depicted in this model: the first (a) is located at the peritubular membrane and the second (b) is an intracellular accumulation site.

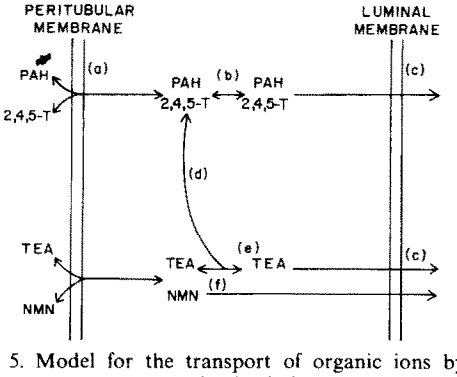

Fig. 5. Model for the transport of organic ions by the proximal tubule.

Movement of PAH occurs via passive diffusion into the lumen (c). This two-step model is supported by a number of studies using renal slices [11, 16–19] and separated tubules [20, 21]. In addition, two component influx and efflux plots for different organic ions have been found [6, 7], and curve stripping of the efflux studies by Huang and Lin [22] also demonstrates a two-component system for PAH. Although all of these data are kinetic in nature and, therefore, do not provide direct experimental evidence for the two transport components, they offer the reasonable suggestion that two components exist.

Due to the high degree of nonspecific binding of 2,4,5-T which is not found for TEA and PAH, a separate step (d) has been added to accommodate this characteristic of 2,4,5-T transport. Also, consideration is given to the observation that 2,4,5-T blocks steady-state TEA uptake (site e), but has no effect on the initial influx of this cation when the 2,4,5-T is added directly to fresh tissue slices [7]. Hence, it is proposed that 2,4,5-T interferes with intracellular TEA accumulation (e), but not the membrane transport step for organic cations. This is consistent with the increased efflux of TEA. That is, TEA is released from intracellular storage by 2,4,5-T and hence is available for more rapid efflux from the cell. The model also suggests that, since 2,4,5-T does not alter NMN efflux, NMN does not have a significant intracellular accumulation or binding site (f). Once NMN is transported into the cell, it is essentially in free solution, and, therefore, the level of NMN uptake is solely a function of the magnitude of the membrane transport step.

In summary, it appears that 2,4,5-T has unique characteristics as an organic anion which permits it to interact with the transport of at least one organic cation, TEA. The important characteristic is probably the high degree of tissue binding noted with 2,4,5-T. In renal cortex homogenates, as much as 50–60 per cent 2,4,5-T binding has been observed in preliminary experiments. With PAH, about 10–15 per cent binding was noted and with phenolsulfophthalein (PSP), about 25 per cent.

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