RENAL TUBULAR EXCRETION OF TRIETHYLCHOLINE (TEC) IN THE CHICKEN: ENHANCEMENT AND INHIBITION OF RENAL EXCRETION OF CHOLINE AND ACETYLCHOLINE BY TEC

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1 [³H]-triethylcholine (TEC) was actively transported by the renal tubule of the chicken at a rate 85% that of simultaneously administered *p*-aminohippuric acid (PAH).

2 TEC was demonstrated to be transported by the organic cation transport system in the kidney through inhibition with quinine and the bio-cation choline.

3 When the infusion of TEC was increased to 2×10^{-6} mol kg⁻¹ min⁻¹ reaching the infused kidney, the transport of [³H]-TEC was inhibited, suggesting that an excretory transport maximum for TEC in the renal tubules had been reached.

4 The excretion of both choline and acetylcholine was enhanced by TEC loads as low as 1×10^{-18} mol kg⁻¹ min⁻¹. Enhancement continued as TEC infusion was increased up to approximately 1×10^{-7} mol kg⁻¹ min⁻¹ at which point this enhancement was converted to inhibition.

5 Possible mechanisms for the biphasic effect of TEC on organic cation transport are discussed.

Introduction

Choline is one of the substrates for acetylcholine synthesis at cholinergic nerve endings and these nerve terminals are known to possess a transport process for the translocation of the choline from the extracellular fluid to the intraneuronal sites of its acetylation. (Potter, 1968; Marchbanks, 1968; Yamamura & Snyder, 1972; Hubbard, 1973). The plasma concentration of choline has been the subject of a number of investigations (Hunt, 1915; Bligh, 1952; 1953) and homeostatic mechanisms maintain a stable plasma choline level (Gardiner & Paton, 1972; Acara & Rennick, 1973).

The proximal renal tubule can actively transport organic cations from the blood to the urine and choline and acetylcholine are examples of bio-cations that are transported by the renal tubule (Rennick, 1958; Acara & Rennick, 1972a,b).

The present experiments were performed to compare the analogue of choline, triethylcholine (triethyl 2-hydroxyethyl ammonium, TEC) with choline in respect to renal transport and metabolism. TEC has been shown to produce neuropharmacological effects at cholinergic nerve terminals which suggest an interaction with endogenous choline (Bowman, Hemsworth & Rand, 1967). The transport of TEC across the renal tubule has not previously been explored and the present experiments were designed to investigate the effect of TEC on the renal tubular transport of choline and acetylcholine.

The pharmacological activity of TEC is similar to that of the hemicholinium, HC-3 (Bowman & Rand, 1961; Bowman *et al.*, 1967). HC-3 has been shown to enhance the excretion of choline in the chicken at an infusion rate of only 1×10^{-22} mol kg⁻¹ min⁻¹ (Acara, Kowalski & Rennick, 1973). In the present experiments TEC was also shown to enhance choline excretion.

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Figure 1 Diagram of renal portal circulation in chicken kidney. Infusion of transportable substrates into right leg flows to the right kidney and enters the portal circulation to bathe only the tubules of the right kidney (From Acara, Kowalski & Rennick, (1973), reproduced by permission of the *Journal of Pharmacology and Experimental Therapeutics*).

Methods

Sperber technique for the determination of active transport in chickens

The Sperber technique for the study of renal tubular transport (Sperber, 1948; Lindahl & Sperber, 1958) takes advantage of a separate renal portal circulation in the chicken, accessible through a leg vein (Figure 1). Urine is collected separately from each ureter and the excretion per minute of substrate from the infused and control kidney is measured. Data are expressed as apparent tubular excretion fraction (ATEF) and are calculated as follows:

$$\text{Exc(Infused)} - \text{Exc(Control)} / \text{Amount infused.}$$

The ATEF thus represents the proportion of the infused substrate actively excreted by the tubules of the infused kidney. In the present experiments the ATEF is expressed as a percentage. To show active transport, an ATEF of greater than 8% is necessary since this percentage could result from diffusion. The ATEF of *p*-aminohippuric acid (PAH) is a measure of the amount of infusion which reaches the infused kidney since PAH is completely extracted by the renal tubules. Transport efficiency (TE) expresses the ratio, ATEF substrate/ATEF (PAH). The TE relates the amount of compound that was transported by the renal tubule to that which reached the kidney.

Collection of urine

Chickens weighing between 2-3 kg were used without anaesthesia and the experimental procedure was that described previously (Acara &

Rennick, 1972a). To maintain an adequate urine flow the chickens were hydrated with 60 ml of water by crop tube at the start of each experiment. Additional loads of 50 ml of water were given every 30 or 40 minutes. During the saline (0.9% w/v NaCl solution) infusion, the ureters were exposed in the cloaca, local anaesthetic applied, and a plastic tube for urine collection was sewn over each ureter. Urine was collected for a period of 10 min and unless otherwise indicated all data represent the mean of three 10 min urine collections.

Infusion solutions

It has been shown previously that choline is actively transported by the renal tubule only at infusion rates greater than $1 \times 10^{-6} \text{ mol kg}^{-1} \text{ min}^{-1}$ reaching the kidney (Acara & Rennick, 1972a). At infusion rates lower than this, choline was extensively metabolized by the renal tubule. A predominant metabolite was betaine which was not transported by the renal tubule. For this reason, the choline infusion rate was kept high for all experiments at $1 \times 10^{-5} \text{ mol/minute}$. A tracer amount of choline-[methyl- ^{14}C] was added to the choline infusion solution. Acetyl-[1- ^{14}C] choline was infused at a rate of $1 \times 10^{-10} \text{ mol kg}^{-1} \text{ min}^{-1}$. All infusion rates were factored by the simultaneous PAH excretion to give values that represent the amount of the infused TEC actually reaching the infused kidney.

Analysis of urine

Aliquots of collected urine were added to Bray's (1960) scintillation solution and counted at

double label settings for 10 min in a Nuclear-Chicago scintillation spectrometer. Correction was made for the crossover of ^{14}C into the tritium channel. Where large concentrations of TEC iodide were infused and thus entered the urine, the colour quenched the ct/min reading in the spectrometer when Bray's solution was used. The substitution of Aquasol (New England Nuclear Corp., Boston, Mass.) was made to prevent any quenching. The ^{14}C -label, as either choline-[methyl- ^{14}C] or acetyl-[1- ^{14}C] choline, was not isolated and identified in urine collections but was assumed to represent the infused compound. Previous studies had given evidence for transport of the intact molecule of choline and acetylcholine (Acara & Rennick, 1972a,b).

Materials

p-Aminohippuric-[1- ^{14}C] acid ([^{14}C]-PAH) 2.52 mCi/mmol and *p*-aminohippuric-[glycyl-2- ^3H] acid ([^3H]-PAH) 205.8 mCi/mmol were obtained from New England Nuclear Corporation, Boston, Mass. Acetyl-[1- ^{14}C] choline chloride, 11.7 mCi/mmol and choline chloride-[methyl- ^{14}C], 54 mCi/mmol were obtained from Amersham/Searle Corporation, Des Plaines, Illinois. One source of TEC was tritium labelled by a catalytic exchange process by New England Nuclear Corp. and was purified by thin layer chromatography by the method of Hemsworth & Bosmann (1971). The purified [^3H]-TEC had a specific activity of 37.1 mCi/mmol. In some experiments [^3H]-TEC, 25 mCi/mmol was used as purchased from New England Nuclear Corp. TEC chloride and TEC iodide were used as carrier.

Results

Renal tubular transport of TEC

The ipsilateral infusion of [^3H]-TEC into a leg vein at rates of 1×10^{-10} to 2×10^{-9} mol kg^{-1} min^{-1} into the peritubular blood of the chicken kidney resulted in active tubular transport of TEC. The apparent tubular excretion fraction (ATEF) for TEC was 52% (Table 1) illustrating that active tubular excretion of TEC does occur. The ATEF of PAH of 60.8% (Table 1) represents the proportion of the infused PAH that reached the renal tubules of the infused kidney. If the ATEF of TEC is compared to the ATEF of PAH, the resulting value, the transport efficiency (TE) for TEC of 0.85 ± 0.07 indicates tubular excretion of 85% of that TEC reaching the infused kidney. This percentage value gives a clearer indication of the

ability of the tubule cells to transport TEC and demonstrates effective tubular transport.

Inhibition of TEC transport by cationic competitors

One of the criteria used commonly to detect active tubular transport is to measure the inhibitory effect of substances that compete for the same transport pathway, in this case, organic cations. The active transport of TEC was challenged by the organic cation quinine, which at a concentration of 2×10^{-7} mol kg^{-1} min^{-1} produced greater than 50% inhibition of TEC transport. The effect of the cation choline on the transport of TEC was also observed and in two experiments 2×10^{-6} mol kg^{-1} min^{-1} choline produced 20% inhibition of the transport of TEC. In five experiments, increasing loads of unlabelled TEC were added during a constant infusion of [^3H]-TEC (from 1×10^{-10} to 2×10^{-9} mol kg^{-1} min^{-1}) (Figure 2). As the TEC load was increased, [^3H]-TEC transport was inhibited. Loads of TEC around 2×10^{-6} mol kg^{-1}

Table 1 Renal tubular transport of triethylcholine (TEC) by the chicken kidney by means of the Sperber technique

Apparent tubular excretion fraction (ATEF ² as %)		Transport efficiency (TE)
[^3H]-TEC ¹	[^{14}C]-PAH ¹	[^3H]-TEC, ATEF / [^{14}C]-PAH, ATEF
62	66	0.94
53	66	0.80
47	58	0.81
52	53	0.98
52	66	0.79
56	60	0.93
32	37	0.86
42	53	0.79
73	82	0.89
40	54	0.74
68	79	0.86
47	55	0.85
—	—	—
Mean 52.00	60.75	0.85
±s.e. ±11.70	±12.21	±0.07

1. Infusion rates: [^{14}C]-PAH at 1.8×10^{-10} mol kg^{-1} min^{-1} , [^3H]-TEC from 1×10^{-10} to 2×10^{-9} mol kg^{-1} min^{-1} .

2. $\text{ATEF} = \frac{\text{ExcInfused kidney} - \text{Exccontrol kidney}}{\text{Rate of infusion}}$

The data on each horizontal line represent an individual chicken experiment.

Figure 2 The effects of infusion of triethylcholine (TEC) at increasing concentrations on the renal tubular excretion of [³H]-TEC. The [³H]-TEC excretion is expressed as % of control excretion rate. [³H]-TEC infusion rate from 1 × 10⁻¹⁰ to 2 × 10⁻⁹ mol kg⁻¹ min⁻¹. All infusion rates were factored by simultaneous *p*-aminohippuric acid excretion to give actual amount reaching infused kidney. *n* = 5.

Figure 3 The effect of infusion of triethylcholine (TEC) at increasing rates on the renal tubular excretion of [¹⁴C]-acetylcholine. The [¹⁴C]-acetylcholine infusion rate was 7.1 × 10⁻¹² to 8.7 × 10⁻¹¹ mol kg⁻¹ min⁻¹. *n* = 5. Conditions as in Figure 2.

min⁻¹ reaching the infused kidney, produced a 70% inhibition of [³H]-TEC transport.

Enhancement of transport of choline and acetylcholine by very low infusion loads of TEC and inhibition by high loads of TEC

The Sperber technique has been used by Acara & Rennick (1972a,b) to demonstrate that both

choline and acetylcholine are actively transported by the renal tubule in the chicken. Table 2 demonstrates the effects of TEC infusions from 1 × 10⁻¹⁸ to 1 × 10⁻⁵ mol kg⁻¹ min⁻¹ reaching the infused kidney.

At a concentration of TEC of 1 × 10⁻¹⁸ mol kg⁻¹ min⁻¹ reaching the infused kidney, choline excretion was potentiated 17%. This enhancement of choline excretion persisted with TEC loads up

Table 2 Effect of triethylcholine (TEC) on the tubular transport of [¹⁴C]-choline in the chicken kidney measured by the Sperber technique

	ATEF % ²		TE	
	[³ H]-PAH ¹	[¹⁴ C]-choline	ATEF [¹⁴ C]-choline ATEF [³ H]-PAH	% of control
Control	67	24	0.36	100
During infusion of TEC (mol kg ⁻¹ min ⁻¹)				
1 × 10 ⁻¹⁸	56	23	0.42	117
1 × 10 ⁻¹³	56	24	0.43	120
1 × 10 ⁻¹⁰	65	28	0.42	117
5 × 10 ⁻⁸	69	24	0.35	85
1 × 10 ⁻⁷	68	24	0.36	88
2.7 × 10 ⁻⁶	60	11	0.19	47
5.5 × 10 ⁻⁶	64	6.4	0.099	24
1 × 10 ⁻⁵	58	3.2	0.056	14

1. Infusion throughout of [³H]-PAH at 2.9 × 10⁻¹¹ mol kg⁻¹ min⁻¹ and [¹⁴C]-choline plus unlabelled choline at 2.8 × 10⁻⁶ mol kg⁻¹ min⁻¹ reaching the infused kidney.
2. As in Table 1.

Figure 4 The effects of infusion of triethylcholine (TEC) at increasing rates on the tubular excretion of $[^{14}\text{C}]$ -choline. The choline infusion rate was 2.3×10^{-6} to $3.2 \times 10^{-6} \text{ mol kg}^{-1} \text{ min}^{-1}$. All other conditions as in Figure 2. $n = 5$.

Figure 5 A comparison of the data from $[^{14}\text{C}]$ -choline ($\bullet$), $[^{14}\text{C}]$ -acetylcholine ($\circ$) and $[^3\text{H}]$ -triethylcholine (TEC) ($\blacksquare$) excretion in the presence of increasing infusions of TEC. Infusion rates as in Figures 2, 3 and 4.

to $5 \times 10^{-8} \text{ mol kg}^{-1} \text{ min}^{-1}$, when a 15% inhibition of choline excretion occurred. Concentrations of TEC greater than $5 \times 10^{-8} \text{ mol kg}^{-1} \text{ min}^{-1}$ produced even more inhibition of choline excretion, an 86% inhibition being observed in the presence of TEC $1 \times 10^{-5} \text{ mol kg}^{-1} \text{ min}^{-1}$.

Table 3 shows similar results on the effect of TEC on the transport of $[^{14}\text{C}]$ -acetylcholine. TEC perfused into the leg vein to reach the kidney at a concentration of $3 \times 10^{-14} \text{ mol kg}^{-1} \text{ min}^{-1}$

produced a 25% enhancement of acetylcholine excretion. This enhancement of acetylcholine excretion continued as the infusion load of TEC was increased up to $2.5 \times 10^{-8} \text{ mol kg}^{-1} \text{ min}^{-1}$. At a higher TEC load the enhancement decreased and at a TEC infusion rate of $2 \times 10^{-6} \text{ mol kg}^{-1} \text{ min}^{-1}$, a 73% inhibition of acetylcholine transport was produced.

Figures 3 and 4 show the results of a number of experiments of the same design as those shown in

Table 3 Effect of triethylcholine (TEC) on the tubular transport of the ^{14}C -label associated with acetylcholine in the chicken kidney measured by the Sperber technique

	ATEF % ²		TE ATEF $[^{14}\text{C}]$ -ACh ATEF $[^3\text{H}]$ -PAH	% of control
	$[^3\text{H}]$ -PAH ¹	$[^{14}\text{C}]$ -acetyl-1 choline		
Control	69	30	0.44	100
During infusion of TEC ($\text{mol kg}^{-1} \text{ min}^{-1}$)				
3×10^{-14}	74	40	0.55	125
3×10^{-12}	72	42	0.58	132
3×10^{-10}	64	36	0.56	127
2.5×10^{-8}	61	34	0.55	125
2×10^{-7}	52	26	0.50	114
2×10^{-6}	47	6	0.12	27

1. Infusion throughout of $[^3\text{H}]$ -PAH at $0.9 \times 10^{-11} \text{ mol kg}^{-1} \text{ min}^{-1}$ and $[^{14}\text{C}]$ -acetylcholine at $3.9 \times 10^{-11} \text{ mol kg}^{-1} \text{ min}^{-1}$.

2. As in Table 1.

Tables 2 and 3. The excretion of [^{14}C]-acetylcholine (Figure 3) and [^{14}C]-choline (Figure 4) in the presence of varying infusion rates of TEC are plotted as a percentage of the control excretion. Infusion rates of TEC as low as $1 \times 10^{-18} \text{ mol kg}^{-1} \text{ min}^{-1}$ reaching the infused kidney were also shown to enhance the excretion of choline and acetylcholine. The biphasic effect of enhancement of excretion of acetylcholine and choline at low loads of TEC and inhibitions at high loads of TEC is apparent from these experiments.

When the data from the choline, acetylcholine and TEC experiments are plotted together (Figure 5) it can be seen that the effects of TEC are quantitatively similar on the transport of all three of these organic cations.

Discussion

TEC was found to be actively transported across the renal tubule in the chicken kidney *in vivo* and the transport of TEC was found to be blocked specifically by the cationic competitor quinine. The rate of TEC transport, expressed as transport efficiency (TE), 0.85 at an infusion rate of TEC of less than $10^{-9} \text{ mol kg}^{-1} \text{ min}^{-1}$ was greater than that for the maximum TE of choline, 0.30 at an infusion rate of $10^{-5} \text{ mol/minute}$. It is possible that TEC is transported more rapidly than choline because it may not be metabolized to any appreciable extent by the kidney whereas choline is metabolized by the kidney to betaine (Acara & Rennick, 1972a). Choline oxidase is the enzyme present in the kidney tubule which metabolizes choline to betaine and this metabolite is not actively transported. Acetylcholine is another renal metabolite of choline which appears in the urine during choline infusion (Acara & Rennick, 1972b). Acetylcholine is actively transported by the renal tubule during acetylcholine infusion.

TEC is not a substrate for choline oxidase (Wells, 1954) and therefore it is probable that TEC is not oxidized to the corresponding aldehyde in the kidney and excreted in this form. However, TEC is acetylated by choline acetyltransferase, the enzyme responsible for acetylcholine synthesis, although the apparent K_m for TEC acetylation is much greater than the apparent Michaelis-Menten constant for choline acetylation (Hemsworth & Smith, 1970). It is possible therefore that some of the TEC in the plasma of the chicken after intravenous infusion may be converted to the acetylated form of TEC and excreted in this form. It is not known whether acetyl TEC is actively transported by the kidney tubule. The present results show that [^3H]-TEC is rapidly excreted and it is suggested that the major portion of this

excreted material is in a non-metabolized form.

At high concentrations TEC inhibits choline and acetylcholine transport through the renal tubule and these effects are similar to those observed on the inhibition of [^{14}C]-choline uptake into synaptosomes and synaptic vesicles (Hemsworth, Darmer & Bosmann, 1971). It is of interest that Acara *et al.* (1973) could find no evidence *in vivo* for inhibition by HC-3 of choline transport across the renal tubule of the unanaesthetized chicken. The unanaesthetized chicken could not tolerate HC-3 at infusion rates greater than $1 \times 10^{-7} \text{ mol/min}$ (Acara *et al.*, 1973) and subsequent experiments with the isolated perfused rat kidney demonstrated that HC-3 was an effective inhibitor of renal tubular transport of choline (Trimble, Acara & Rennick, 1974).

HC-3 is generally considered to be the most potent of the drugs which inhibit choline transport. However, although TEC possesses a more marked ganglion blocking action than HC-3 (Bowman & Rand, 1961) the respiratory depression caused by TEC is less than that occurring with HC-3. TEC is therefore less toxic than HC-3 and would appear to be the better choice to investigate choline transport *in vivo*.

The present experiments illustrate that very small loads of TEC enhance the excretion of choline or acetylcholine. As the load of TEC was increased beyond $5 \times 10^{-7} \text{ mol kg}^{-1} \text{ min}^{-1}$ reaching the kidney, a reversal of the response was seen and the excretion of both choline and acetylcholine was inhibited, suggesting a competitive inhibition of tubular transport of these cations by TEC. This represents a biphasic response of the tubular mechanisms. Similar enhancing effects on choline and acetylcholine excretion were seen with HC-3 at similarly low loads (Acara *et al.*, 1973). A likely explanation for the enhancement of excretion of choline or acetylcholine caused by low loads of HC-3 or TEC may relate to the proposed bidirectional transport of choline. In a manner analogous to uric acid transport, choline may be transported actively in both the direction of excretion and reabsorption (Acara & Rennick, 1973). The two directions of transport probably occur simultaneously and the rate of transport in the two directions and the tubular transport maxima (T_m) may be unequal. One direction of the transport may be sensitive to inhibition by lower concentrations of the cationic competitors. In the case of HC-3 and TEC, the reabsorptive transport may be the more sensitive to the low concentrations and hence the net excretion of choline would be enhanced. When high loads of TEC are offered, both reabsorption and excretory transports may be blocked, resulting in a reduced excretion of choline.

HC-3 also does not possess a very potent anticholinesterase action (Domino, Shellenberger & Frappier, 1968; Hemsworth, 1971) again suggesting that anticholinesterase activity does not play any great role in the potentiation of acetylcholine transport by both TEC and HC-3. Both TEC and HC-3, at low concentrations, have previously been reported to enhance the uptake of choline into synaptosomes and synaptic vesicles *in vitro* whereas at higher concentrations of these choline analogues the uptake of choline was inhibited (Hemsworth *et al.*, 1971). It was suggested that both TEC and HC-3 might themselves be transported across the synaptosomal membrane and it is of interest that the present results show that TEC can be transported across the renal tubule by a choline like transport process. It is also of interest that Gomez, Domino & Sellinger (1969) showed that HC-3 caused an increase in choline uptake into dog caudate nucleus *in vivo*. Also Sellinger, Domino, Haarstad & Mohrman (1969) and Rodriguez de Lores Arnaiz, Zieher & de Robertis (1970) showed that HC-3 can be taken up into synaptosomes and synaptic vesicles *in vitro*. It is apparent therefore that both TEC and HC-3 can be transported by the choline transport system. In this respect Hemsworth & Bosmann (1971) have shown that [^3H]-TEC is taken up into guinea-pig synaptosomes and synaptic vesicles *in vitro*.

Other workers have demonstrated both enhancement and inhibition of transport by different concentrations of drugs. Holm (1971; 1972) investigated the uptake of some quaternary ammonium compounds and showed that the

transport of decamethonium into mouse kidney slices was inhibited by high concentrations of carbamoylcholine, choline, neostigmine and tetraethylammonium, whereas lower concentrations of these drugs potentiated the uptake of decamethonium.

Levine, Oxender & Stein (1965) and Heinz & Walsh (1958) have reported on studies concerning the acceleration of the transport of various hexoses and amino acids. The enhancement of choline and acetylcholine by TEC could be an example of accelerative exchange diffusion or could be due to substrate facilitated carrier transport; however, it seems likely that the carrier mechanism for both choline and acetylcholine and for TEC involves a common quaternary nitrogen carrier mechanism.

The enhancement of excretion of choline and acetylcholine *in vivo* by these small loads of TEC in the present Sperber chicken experiments may result from either an increase in tubular excretion or an inhibition of tubular reabsorption. At the same time the small loads of TEC may alter the intracellular metabolism and disposition of choline and acetylcholine.

The inhibition of excretion of choline and acetylcholine produced by high loads of TEC is probably a result of competition by the TEC for the common organic base carrier which transports choline and acetylcholine across the renal tubule in the direction of excretion.

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