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INHIBITION OF RENAL TUBULAR TRANSPORT OF MORPHINE BY DIETHYLAMINOETHANOL IN THE CHICKEN*

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Abstract—Using the model system developed for renal tubular transport and metabolism, the morphine–morphine-3-ethereal sulfate (MES) system in the Sperber preparation of the chicken, diethylaminoethanol (DEAE) and possibly Lilly 18947 (2,4-dichloro-6-phenylphenoxyethyldiethylamine) were shown to inhibit morphine transport. Because this inhibition with DEAE led to decreased access of morphine into the renal tubular cell, the excretion of morphine and MES in the urine decreased. However, the results of countercurrent distribution analyses indicated that the amount of MES relative to morphine did not decrease and, if anything, increased. Therefore, DEAE did not inhibit the metabolism of morphine to MES. Transport of ^{14}C-tetraethylammonium was also inhibited; transport of ^{14}C-MES was not altered by DEAE. Since Lilly 18947 reduced the apparent tubular excretion fraction of PAH and of administered ^{14}C-MES, conclusions about the site of action of Lilly 18947 were not as firmly established. DEAE and possibly Lilly 18947 appeared to block morphine transport by virtue of their highly basic property. The fact that diethylaminoethanol occurs as a chemical moiety in these as well as in other compounds gives rise to the expectation that a number of compounds containing this moiety should show the same type of blocking activity on the organic cation transport system.

IN A PREVIOUS publication,[1] we reported that β-diethylaminoethyldiphenylpropylacetate (SKF 525A) and *N*-methyl-3-piperidyl-*N'*,*N'*-diphenylcarbamate (MPDC) blocked the access of morphine into the renal tubular cell in the experiments *in vivo* with the Sperber preparation of the chicken. The attributes of this preparation, as developed for the study of the transport and metabolism of morphine,[1–5] made it possible to show clearly that SKF 525A and MPDC inhibited transport of morphine but did not inhibit the conjugation of morphine with sulfuric acid to form morphine-3-ethereal sulfate. Furthermore, this effect of SKF 525A and MPDC was shown to be due to their action as organic bases to block the organic cationic transport system. Therefore, this effect extended to blocking the transport of tetraethylammonium.

In the present study, Lilly 18947 (2,4-dichloro-6-phenylphenoxyethyldiethylamine) and diethylaminoethanol (DEAE) will be tested to see whether these compounds act like SKF 525A and MPDC on morphine transport. Lilly 18947 was included because, like SKF 525A, it is used as an inhibitor in drug metabolism studies.[6,7] DEAE is a common chemical moiety which occurs not only in SKF 525A but also in Lilly 18947 as well as in CFT 1201 (diethylaminoethyl-2,2-diallyl-2-phenylacetate) and Sch 5712 (ethyl, diethylaminoethyl-2-butyl-2-ethylmalonate), which are also microsomal enzyme inhibitors.[6,7] Because DEAE is the nitrogen-containing portion which confers the

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organic base property to all of these compounds, the present studies do give an insight into why many, if not all, such compounds will be inhibitors of the organic cationic transport system in the kidney.

METHODS

Renal tubular transport and metabolism of morphine were investigated in unanesthetized Rhode Island Red laying hens weighing 2·9–3·5 kg. They were prepared as described in the previous publication with SKF 525A.[1] Plastic tubing with sponge rubber cuffs was sutured on the ureteral orifices for urine collection. The tubing was rinsed continuously at a rate of 0·25 ml/min with distilled water. Urine samples and rinse were collected for 10-min periods; the final volume of each sample was brought up to 10 ml by adding distilled water.

The control and test solutions for the intravenous infusion (rate of infusion = 0·42 ml/min) into the saphenous vein were made up in serial fashion. *p*-Aminohippuric acid (PAH), 16 mg, was dissolved in 200 ml of 0·9% sodium chloride. For the control infusion solution, *N*-$^{14}CH_3$-morphine hydrochloride (57 mc/m-mole), 0·3 to 1 ml stock solution of 10 μc/ml, was added to 125 ml of the PAH solution. This control solution gave ^{14}C counting ranges of 2623–6335 counts/min/0·1 ml. In each experiment, the infusion solution was counted to determine the exact ^{14}C infusion rate. The ^{14}C-morphine was obtained from Amersham/Searle. For the test infusion solution, either diethylaminoethanol (DEAE) or Lilly 18947 was dissolved in 42 ml of the control infusion solution. The doses of diethylaminoethanol and Lilly 18947 are given in the tables with the results.

In other experiments, either tetraethyl-1-[^{14}C]-ammonium (TEA) bromide, 3 mc/m-mole infused at 0·009 μc/min (obtained from New England Nuclear Corp.), or *N*-$^{14}CH_3$-morphine-3-ethereal sulfate (MES, 3·5 mc/m-mole infused at 0·003 μc/min) was used in place of the ^{14}C-morphine and the effect of diethylaminoethanol and Lilly 18947 were assessed. The ^{14}C-MES was made biosynthetically by administering ^{14}C-morphine to the cat and isolating the crystalline metabolite as described previously.[8,8]

The experimental protocol was to infuse the ^{14}C control solution (morphine, MES or TEA) for 85–105 min before timed urine collections were started. This period allowed the system to come to a steady state. Then, 10-min control urine samples were collected simultaneously from both kidneys for five such periods. Subsequently, the intravenous infusion solution was switched to the test solution which included either the DEAE or Lilly 18947 in the control infusion solution. Ten-min urine samples were collected as before; seven or eight periods of collection were made.

Total ^{14}C-radioactivity was determined by placing 0·1 ml of infusion solution or 0·2 ml of diluted urine solution in 15 ml of scintillation fluid prepared with 4 g 2,5-diphenyloxazole (PPO) and 50 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in a mixture of 1000 ml toluene and 500 ml Triton X-100. Details of the counting procedure were as described previously.[1] PAH was determined colorimetrically by taking 0·5 ml of the diluted urine sample.[1]

The apparent tubular excretion fraction (ATEF) was calculated in the same fashion as previously[1] where

$$\frac{EXC_I - EXC_C}{INF} \times 100 = ATEF$$

EXC_I was the amount excreted in the urine from the infused side; EXC_C was the amount excreted from the contralateral side, and INF was the amount infused during each 10-min period. The infusion volume, dilution and sampling parameters chosen were such that ATEF for ^{14}C could be calculated where the counts in 0·2 ml of diluted urine and 0·1 ml of ^{14}C infusion solution could be inserted into the above equation to calculate the ATEF.

In all experiments where ^{14}C-morphine was infused, the total radioactivity in the urine samples from the infused side was fractionated by countercurrent distribution. This fractionation enabled quantitation of ^{14}C-morphine and its metabolite, morphine-3-ethereal sulfate. Two ml of diluted urine was adjusted to pH 8·5 with concentrated ammonium hydroxide with a microsyringe. The countercurrent distribution system consisted of 2 ml of each phase of a $KHCO_3$ (2 g/l) buffer, pH 8·5 and chloroform (with 1%, v/v, *N*-butanol) in an 8-transfer manual operation as described previously.[1] Chloroform was the mobile phase. The amount of MES was estimated by summing up the radioactivity in tubes 0–3. The amount of morphine was calculated as the total radioactivity in the countercurrent system minus that of the MES.

Significance of the differences between mean values was assessed by the *t*-test and, even though P values less than 0·01 were obtained in many cases, $P \leq 0{\cdot}05$ was taken as the level of significance.

RESULTS

Effects of Lilly 18947 *on morphine and PAH transport.* Table 1 shows the data for the ATEF of ^{14}C-morphine and PAH where high ^{14}C and PAH ATEF values during control periods indicate active transport.[5] Profound decreases in ^{14}C ATEF (all P values $\leq 0{\cdot}05$) occurred when Lilly 18947 was infused at 0·2 to 0·5 mg/kg/min. The ATEF for PAH also fell significantly ($P \leq 0{\cdot}05$) in two of the three experiments. However, this fall was not as great as the fall in the ^{14}C ATEF. The fall in PAH ATEF seen with SKF 525A and MPDC previously[1] was ascribed to opening of the venous bypass valve in the renal portal system. By analogy, the effect of Lilly 18947 on the PAH ATEF may be on the venous bypass valve also. The quantitatively much greater drop in ^{14}C than in PAH ATEF must be due to an additional effect. The presence of this additional effect is readily seen by examining the ^{14}C/PAH ATEF ratio in Table 1. In all cases, Lilly 18947 caused a differentially greater fall in ^{14}C than in PAH ATEF, as seen by the fall in the ratio ($P \leq 0{\cdot}05$).

The last column in Table 1 gives the results of the countercurrent analysis of the urine samples from the infused side. Note that the results are given in terms of the per cent of ^{14}C-radioactivity in the urine sample appearing as ^{14}C-MES. Since the total ^{14}C excretion was decreased by Lilly 18947 as given by the ^{14}C ATEF, the purpose of fractionating the excreted ^{14}C into MES and morphine was to see whether this decrease in ^{14}C excretion might be due to a decrease in the excretion of ^{14}C-morphine or ^{14}C-MES or both. If one expects Lilly 18947 to inhibit only the metabolism of morphine, then the per cent ^{14}C-MES must decrease. The data show that the per cent ^{14}C-MES does not decrease; the per cent ^{14}C-MES increased ($P \leq 0{\cdot}05$). Thus, the results are not compatible with any postulate involving inhibition of metabolism of morphine to MES. The hypothesis compatible with the results is that Lilly 18947, like SKF 525A and MPDC, may be inhibiting transport of ^{14}C-morphine into the renal tubular cell. In other words, the fall in ^{14}C ATEF is due not only to opening of the

TABLE 1. EFFECT OF LILLY 18947 ON ^{14}C-MORPHINE AND PAH TRANSPORT

Expt. No.	Treatment drug	Dose (mg/kg/min)	No. 10-min periods	Apparent tubular excretion fraction*			% of ^{14}C as ^{14}C-MES†
				^{14}C	PAH	^{14}C/PAH	
P48	Control		5	40·0 ± 2·4	52·6 ± 2·4	0·76 ± 0·03	65·2 ± 0·9
	18947	0·5	7	5·5 ± 2·3‡	22·9 ± 5·1‡	0·25 ± 0·05‡	82·6 ± 2·3‡
P52	Control		5	51·7 ± 1·4	85·0 ± 2·5	0·60 ± 0·03	53·6 ± 2·7
	18947	0·3	8	13·9 ± 1·3‡	53·6 ± 7·0‡	0·23 ± 0·02‡	76·4 ± 2·9‡
P62	Control		5	60·0 ± 0·6	90·4 ± 4·7	0·67 ± 0·03	62·5 ± 1·6
	18947	0·2	8	21·2 ± 2·4‡	80·8 ± 2·5	0·27 ± 0·03‡	79·0 ± 1·4‡

* Values given are means ±S.E. Apparent tubular excretion fraction (ATEF) was calculated as given in Methods.

† Values given are means ±S.E. Countercurrent analysis was performed on each urine sample from the infused side and MES was measured as the ^{14}C occurring in tubes 0–3; this ^{14}C expressed as a percentage of the total ^{14}C present in the system gave per cent ^{14}C-MES. The per cent ^{14}C-morphine would be 100 − per cent ^{14}C-MES.

‡ Mean value for control vs drug treatment was significantly different ($P \leq 0.05$) by the Student's *t*-test.

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TABLE 2. EFFECT OF DIETHYLAMINOETHANOL (DEAE) ON ^{14}C-MORPHINE AND PAH TRANSPORT

Expt. No.	Treatment drug	Dose (mg/kg/min)	No. 10-min periods	Apparent tubular excretion fraction*			% of ^{14}C as ^{14}C-MES†
				^{14}C	PAH	^{14}C/PAH	
P28	Control		5	28·1 ± 2·1	57·5 ± 2·3	0·49 ± 0·03	56·1 ± 2·7
	DEAE	0·5	8	15·5 ± 0·6‡	63·4 ± 3·2	0·25 ± 0·02‡	75·8 ± 1·1‡
P32	Control		5	22·9 ± 3·3	51·2 ± 9·2	0·49 ± 0·07	65·3 ± 1·2
	DEAE	0·5	8	11·6 ± 1·1‡	63·2 ± 2·1	0·18 ± 0·02‡	76·5 ± 1·5‡
P72	Control		5	33·0 ± 2·5	43·4 ± 3·4	0·76 ± 0·02	48·9 ± 1·2
	DEAE	0·5	8	5·2 ± 1·6‡	31·2 ± 4·6	0·23 ± 0·09‡	67·1 ± 2·4‡
P75	Control		5	33·8 ± 6·1	44·4 ± 8·9	0·80 ± 0·05	68·0 ± 1·2
	DEAE	0·5	8	16·0 ± 1·3‡	42·0 ± 1·8	0·38 ± 0·03‡	75·3 ± 2·5‡

* Values given are means ±S.E. Apparent tubular excretion fraction (ATEF) was calculated as given in Methods.

† Values given are means ±S.E. Countercurrent analysis was performed on each urine sample from the infused side and MES was measured as the ^{14}C occurring in tubes 0–3; this ^{14}C expressed as a percentage of the total ^{14}C present in the system gave per cent ^{14}C-MES. The per cent ^{14}C-morphine would be 100 − per cent ^{14}C-MES.

‡ Mean value for control vs drug treatment was significantly different ($P \leq 0{\cdot}05$) by the Student's *t*-test.

venous bypass valve but is due additionally to block of ^{14}C morphine transport by Lilly 18947. These points will be pursued further in the Discussion.

Effects of diethylaminoethanol on morphine and PAH transport. Table 2 shows the results with DEAE which in a dose of 0·5 mg/kg/min reduced the ^{14}C ATEF during ^{14}C-morphine infusion. In contrast to Lilly 18947, DEAE had no effect on the ATEF of PAH ($P > 0·05$). Thus, the complicating effect of opening of the venous valve has been circumvented and the drop in ^{14}C ATEF indicates an effect on the morphine system alone. Therefore in all four cases there is a consistent fall in the ^{14}C-PAH/ATEF ratio ($P \leq 0·05$) produced by DEAE. The countercurrent analysis results in Table 2, last column, show that DEAE does not inhibit the metabolism of morphine because the per cent ^{14}C-MES did not drop. In fact, the per cent MES increased in all cases ($P \leq 0·05$). These results taken together with the fact that ^{14}C ATEF was reduced by DEAE were interpreted to indicate that DEAE inhibited transport but not metabolism of ^{14}C-morphine.

Effect of Lilly 18947 *and diethylaminoethanol on ^{14}C-TEA transport.* Since the evidence up to this point indicated that both Lilly 18947 and DEAE appeared to be inhibiting transport of morphine, it was highly likely that such an inhibitory effect of Lilly 18947 and DEAE would extend to transport of bases other than morphine. TEA is known to be transported by the same cation system that transports morphine;[1,4] therefore, the effect of these compounds on TEA transport was studied.

Table 3 shows that Lilly 18947 produced significant reductions in the ^{14}C-TEA to PAH ATEF ($P \leq 0·05$) where in both experiments the ratios were drastically reduced. Therefore, Lilly 18947 seems to block the transport of ^{14}C-TEA.

TABLE 3. EFFECT OF LILLY 18947 ON ^{14}C-TETRAETHYLAMMONIUM (TEA) AND PAH TRANSPORT

Expt. No.	Treatment drug	Dose (mg/kg/min)	No. 10-min periods	Apparent tubular excretion fraction		
				^{14}C-TEA	PAH	^{14}C-TEA/PAH
P84	Control		5	26·1 ± 2·6	11·3 ± 2·0	2·69 ± 0·55
	18947	0·3	8	4·5 ± 4·2*	18·9 ± 2·3*	0·28 ± 0·26*
P104	Control		5	38·8 ± 2·6	32·3 ± 2·4	1·20 ± 0·15
	18947	0·3	7	1·5 ± 2·8*	32·6 ± 7·1	0·07 ± 0·07*

* Mean value for control vs Lilly 18947 treatment was significantly different ($P \leq 0·05$) by the Student's *t*-test.

Table 4 shows similar experiments in which DEAE was used in place of Lilly 18947. In these experiments, DEAE unequivocally reduced the ^{14}C-TEA ATEF ($P \leq 0·05$) in the absence of any reduction in PAH ATEF (in experiment P96, the PAH ATEF was significantly raised above the control value ($P < 0·05$). The significant reduction in all cases of the ^{14}C-TEA/PAH ratio ($P \leq 0·05$) indicated that DEAE blocked transport of ^{14}C-TEA.

Effect if Lilly 18947 *and diethylaminoethanol on ^{14}C-MES transport.* The possible effect of Lilly 18947 and DEAE on the transport of ^{14}C-MES was tested. Table 5 shows that in three of the four experiments, Lilly 18947 reduced the ^{14}C-MES ATEF ($P \leq 0·05$). In these same three experiments, there was a tendency for the PAH ATEF

TABLE 4. EFFECT OF DIETHYLAMINOETHANOL (DEAE) ON ^{14}C-TEA AND PAH TRANSPORT

Expt. No.	Treatment drug	Dose (mg/kg/min)	No. 10-min periods	Apparent tubular excretion fraction		
				^{14}C-TEA	PAH	^{14}C-TEA/PAH
P94	Control		5	59·3 ± 1·9	48·8 ± 3·7	1·13 ± 0·03
	DEAE	0·5	8	27·1 ± 1·3*	46·9 ± 1·3	0·58 ± 0·02*
P96	Control		5	43·7 ± 5·7	28·6 ± 5·2	1·60 ± 0·10
	DEAE	0·5	8	4·4 ± 0·7*	44·1 ± 1·5*	0·10 ± 0·02*
P100	Control		5	35·0 ± 2·6	28·4 ± 1·9	1·24 ± 0·06
	DEAE	0·5	8	13·7 ± 1·4*	27·7 ± 1·7	0·50 ± 0·06*

* Mean value for control vs DEAE treatment was significantly different ($P \leq 0·05$) by the Student's *t*-test.

to fall also. The ratio of the ^{14}C-MES/PAH ATEF in all three cases tended to be depressed. Considering all four experiments, it is difficult to state whether Lilly 18947 had any consistent effect on ^{14}C-MES transport.

TABLE 5. EFFECT OF LILLY 18947 ON ^{14}C-MES AND PAH TRANSPORT

Expt. No.	Treatment drug	Dose (mg/kg/min)	No. 10-min periods	Apparent tubular excretion fraction		
				^{14}C-MES	PAH	^{14}C-MES/PAH
P112	Control		5	41·8 ± 0·05	53·1 ± 3·4	0·79 ± 0·04
	18974	0·3	8	22·9 ± 0·19*	40·5 ± 5·0	0·53 ± 0·11*
P114	Control		5	60·1 ± 0·1	66·8 ± 0·1	0·90 ± 0·02
	18947	0·5	8	26·3 ± 0·2*	33·4 ± 0·2*	0·75 ± 0·08
P116	Control		5	43·1 ± 2·8	47·7 ± 2·5	0·91 ± 0·06
	18947	0·3	8	31·1 ± 1·7*	36·7 ± 5·7	0·75 ± 0·04*
P118	Control		5	71·0 ± 2·4	86·2 ± 7·5	0·85 ± 0·07
	18947	0·2	8	72·7 ± 1·2	92·3 ± 2·4	0·79 ± 0·03

* Mean value for control vs Lilly 18947 treatment was significantly different ($P \leq 0·05$) by the Student's *t*-test.

In contrast to these results, the experiments in Table 6 clearly show that DEAE did not have any effect on ^{14}C-MES transport. In all cases, no significant change in the ratio of the ^{14}C-MES/PAH ATEF was found. In the one case (experiment P110) where the ^{14}C-MES ATEF was reduced by DEAE, there was a corresponding decrease in PAH ATEF so that the ratio of the ATEF values was unchanged in going from control to DEAE treatment. Therefore DEAE had no effect on ^{14}C-MES transport.

DISCUSSION

A brief review of the morphine–MES renal tubular transport and metabolism model system is necessary in order to interpret fully the present experiments. Morphine is transported by the renal tubular system that actively secretes organic cations.[4,5] It is metabolized within the renal tubular cell to morphine-3-ethereal sulfate;[4,5,8] this metabolism can be inhibited by catechol, thereby depressing the appearance of MES

TABLE 6. EFFECT OF DIETHYLAMINOETHANOL (DEAE) ON ^{14}C-MES AND PAH TRANSPORT

Expt. No.	Treatment drug	Dose (mg/kg/min)	No. 10-min periods	Apparent tubular excretion fraction		
				^{14}C-MES	PAH	^{14}C-MES/PAH
P106	Control		5	29·0 ± 15·6	25·4 ± 10·15	1·08 ± 0·08
	DEAE	0·5	8	19·9 ± 6·0	15·7 ± 5·9	1·16 ± 0·11
P108	Control		5	42·4 ± 3·1	38·1 ± 3·1	1·12 ± 0·06
	DEAE	0·5	8	39·6 ± 2·0	41·1 ± 1·4	0·97 ± 0·04
P110	Control		5	64·9 ± 2·3	57·9 ± 1·5	1·12 ± 0·01
	DEAE	0·5	7	55·2 ± 2·7*	49·5 ± 1·9*	1·12 ± 0·06

* Mean value for control vs DEAE treatment was significantly different ($P \leq 0{\cdot}05$) by the Student's t-test.

in the ipsilateral urine samples. At the same time, an expected compensatory increase in free ^{14}C-morphine excretion occurs while the total ^{14}C ATEF remains unchanged.[2] MES, which is administered by i.v. infusion, is transported by the system that secretes organic anions.[4] Probenecid, a compound which blocks the anion transport system, blocks the transport of this administered MES but does not block excretion of MES, which is formed intracellularly by metabolism of morphine to MES. Therefore, a site of action of probenecid is placed at the peritubular border of the renal tubular cell.[4] Many of these general concepts have been further substantiated by experiments with the renal tubular transport and metabolism studies with the serotonin–5-hydroxyindoleacetic acid model system.[3] Reviews of these studies are available.[9,10] In a further paper, a site of action of SKF 525A and *N*-methyl-3-piperidyl-*N'*,*N'*-diphenylcarbamate (MPDC) was shown to be on the transport of morphine and not on the metabolism of morphine in the kidney.[1]

The present experiments indicate that DEAE blocks the organic cation transport system of the renal tubular cell (as did SKF 525A and MPDC[1]). The evidence for this statement is derived from several different experiments. First, DEAE reduces the ^{14}C ATEF during ^{14}C-morphine infusion in the absence of any effect on PAH transport. Second, this reduction in ^{14}C ATEF results from a fall in the concentration of both ^{14}C-morphine and ^{14}C-MES in the urine. Since the relative proportion of ^{14}C-MES to ^{14}C-morphine is larger during DEAE infusion than during control periods without DEAE, there exists no evidence that DEAE inhibits metabolism of morphine to MES. Third, DEAE does not inhibit the transport of ^{14}C-MES that was administered; therefore, it is highly unlikely that intracellularly formed MES excretion would have been blocked. Fourth, DEAE blocks the transport of ^{14}C-TEA without affecting PAH transport. Thus, we conclude that DEAE blocks morphine transport by virtue of its ability to block the organic cation transport system. One probable location of the cation transport system appears to be on the peritubular side.[3]

Certain other possibilities were eliminated by making the following considerations. In the face of a decreased delivery of ^{14}C-morphine from the peritubular fluid to the intracellular compartment, one might question whether in the present experiments we would have been able to detect inhibition of metabolism if it were present simultaneously with the reduction in morphine delivery. Inhibition of metabolism of morphine to MES within the renal tubular cells is known to lead to a compensatory

increase in the proportion of morphine relative to MES, as cited above on the work with catechol. The present results clearly show that the proportion of ^{14}C-MES increased and ^{14}C-morphine fell in the presence of DEAE; these results strongly suggest that DEAE could not have been simultaneously inhibiting formation of MES. The reason for the rise in per cent MES is not known. Since TEA is not metabolized during its transtubular transport, the block of its transport by DEAE demonstrates that block of transport by DEAE can occur independently of any possible effect on metabolism of the compound being transported.

The same arguments developed for DEAE can be developed for the action of Lilly 18947 on morphine and TEA transport. However, the supporting experimental evidence is not so strong as that for DEAE, since Lilly 18947 has several additional effects which complicate the interpretation of the results. Namely, Lilly 18947 has a tendency to open the venous bypass valve of the kidney, as indicated by the fall in PAH ATEF. However, Lilly 18947 causes a much greater fall in both ^{14}C-morphine and ^{14}C-TEA ATEF values than in the PAH ATEF, so that Lilly 18947 has blocking effects on the organic cation transport system beyond that explicable by opening of the venous bypass valve. This interpretation is consistent with the previous results on SKF 525A and MPDC.[1] A further complication is that Lilly 18947 does appreciably block transport of ^{14}C-MES administered intravenously. Yet, when the MES is formed from morphine within the renal tubular cell, Lilly 18947 does not appear to block ^{14}C-MES egress from the cell (Table 1). These two types of MES experiments could be interpreted to mean that Lilly 18947 has some blocking activity against an organic anion transport system known to be located on the peritubular membrane.[3,4] SKF 525A and MPDC were reported previously not to block transport of administered or intracellularly formed MES.[1] By analogy with the latter compounds, the evidence, although weak, is compatible with Lilly 18947 acting to block the transport of organic cations with no inhibition of metabolism of morphine.

As stated in the Introduction, diethylaminoethanol is the portion of the molecule of SKF 525A, Lilly 18947, CFT 1201 and Sch 5712 which confers basic properties to these compounds. Knowing that SKF 525A, diethylaminoethanol itself, and possibly Lilly 18947 block organic cation transport, it would be predicted that CFT 1201 and Sch 5712 should also do the same. Furthermore, other classes of drugs which also contain the diethylaminoethanol moiety, such as procaine, trasentine, and benactyzine, might also block cation transport.

A very interesting study was reported by Foster *et al.*[11] on inhibition of hepatic microsomal enzymes by *N*-substituted ethanolamines. The ethanolamines including diethylaminoethanol were effective microsomal enzyme inhibitors when administered *in vivo*. Since broad specificity exists for substrates and inhibitors of microsomal mixed-function oxidases and broad specificity occurs in substrate transport and inhibitor action in the kidney, it is conceivable that the basic mechanism for interaction of certain substrates and inhibitors on the microsomal and renal transport systems might be very similar. The effect of SKF 525A on membranes was discussed in a previous publication.[1]

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REFERENCES

1. R. HAKIM and J. M. FUJIMOTO, *Biochem. Pharmac.* **20,** 2647 (1971).
2. W. M. WATROUS and J. M. FUJIMOTO, *Biochem. Pharmac.* **20,** 1479 (1971).
3. R. HAKIM, W. M. WATROUS and J. M. FUJIMOTO, *J. Pharmac. exp. Ther.* **175,** 749 (1970).
4. W. M. WATROUS, D. G. MAY and J. M. FUJIMOTO, *J. Pharmac. exp. Ther.* **172,** 224 (1970).
5. D. G. MAY, J. M. FUJIMOTO and C. E. INTRURISI, *J. Pharmac. exp. Ther.* **157,** 626 (1967).
6. A. H. CONNEY and J. J. BURNS, *Adv. Pharmac.* **1,** 31 (1962).
7. M. W. ANDERS, *A. Rev. Pharmac.* **11,** 37 (1971).
8. J. M. FUJIMOTO and V. B. HAARSTAD, *J. Pharmac. exp. Ther.* **165,** 45 (1969).
9. J. M. FUJIMOTO, in *Narcotic Drugs: Biochemical Pharmacology* (Ed. DORIS H. CLOUET), p. 366. Plenum Press, New York (1971).
10. B. R. RENNICK, *A. Rev. Pharmac.* **12,** 141 (1972).
11. G. V. FOSTER, JR., R. HARTUNG and H. H. CORNISH, *Toxic. appl. Pharmac.* **19,** 386 (1971).