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INHIBITION OF RENAL TUBULAR TRANSPORT OF MORPHINE BY β-DIETHYLAMINOETHYL DIPHENYLPROPYLACETATE IN THE CHICKEN*

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Abstract—The Sperber preparation in the chicken was used to study the effects of β-diethylaminoethyl diphenylpropylacetate (SKF 525A) and *N*-methyl-3-piperidyl-*N'*,-*N'*-diphenylcarbamate (MPDC) on the morphine–morphine ethereal sulfate system. Both SKF 525A and MPDC had the pharmacologic effect of opening the venous valve, which caused more blood to bypass the peritubular capillary network of the kidney. This effect was manifested by a decrease in the apparent tubular excretion fraction (ATEF) for *p*-aminohippuric acid. In spite of the presence of this effect, SKF 525A and MPDC produced a large fall relative to PAH in the ATEF for ^{14}C excretion during [^{14}C]-morphine infusion. The results meant that SKF 525A and MPDC blocked the access of [^{14}C]morphine into the renal tubular cell. Because of the presence of this block, excretion of [^{14}C]morphine and [^{14}C]morphine ethereal sulfate decreased. In this situation, the decrease in metabolite formation was not due to inhibition of metabolism, but was the result of inhibition of transport of morphine into the cell. Since this block extends to tetraethyl-1-[^{14}C] ammonium transport, both SKF 525A and MPDC act on the cationic transport system. The implications of this finding deserve emphasis. It was further demonstrated that [^{14}C]pentobarbital was neither transported nor metabolized by the kidney. But, SKF 525A and MPDC did inhibit metabolism of [^{14}C]pentobarbital, metabolism which was taking place in organs other than the kidney.

HARGREAVES[1] reported that β-diethylaminoethyl diphenylpropyl-acetate (SKF 525A) inhibited excretion of certain compounds into the bile at the same time that metabolism of the compounds was inhibited in the liver. Therefore, he suggested that in the liver a close link occurred between metabolism and transport of certain compounds. Earlier work by numerous investigators had shown that SKF 525A inhibited a variety of enzymatic reactions such as *N*-demethylation, deamination, hydroxylation, glucuronide formation, side chain oxidation and ether cleavage.[2–6] Also, metabolites of SKF 525A, among them 2,2-diphenylvalerate (SKF 2314), have been identified and shown to possess inhibitory activity.[7,8] Since there exists a tendency for congruence to occur in hepatic and renal functions,[9] the present investigation was undertaken to study the effect of SKF 525A on metabolism and transport of morphine in the kidney. Another inhibitor, *N*-methyl-3-piperidyl-*N'*,*N'*-diphenylcarbamate (MPDC) was included in the study because it possesses some of the same inhibitory activity that SKF 525A possessed on the liver.[10,11]

The primary system under study, the morphine–morphine ethereal sulfate (MES) system, is unique in that the phenomena of transport and intracellular metabolism of morphine can be delineated in a preparation *in vivo* in the kidney of the chicken.

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Study of the morphine–MES system was initiated by May *et al.*[12] Details of its characteristics were refined by isolation of the metabolite, MES;[13] the transport system for MES itself was defined by Watrous *et al.*[14] Recently, Watrous and Fujimoto[15] showed that metabolism of morphine was inhibited by catechol without any effect on transport of morphine. Thus, the purpose of the present study was to see whether SKF 525A, MPDC and possibly SKF 2314 would inhibit the metabolism of morphine without effects on transport of morphine. The results are particularly interesting since exactly the opposite was found. These agents inhibited the transport of morphine without any effect on the metabolism of morphine to MES. It is suggested that SKF 525A and MPDC produce this inhibition by acting on the cationic organic base transport system. A few experiments were done for comparative purposes, using pentobarbital in place of the primary system under study.

METHODS

Renal tubular transport and metabolism of morphine were studied in unanesthetized Rhode Island Red laying hens (2·2–3·0 kg). They were prepared according to the technique described by Sperber[16] and presently in use in this laboratory.[15,17] Plastic tubes with sponge rubber cuffs were sutured on the ureteral orifices for urine collection. The tubes were rinsed continuously at a rate of 0·246 ml/min with sodium bicarbonate buffer (2 g/l.), pH 8·4–8·6, to avoid clogging with uric acid as well as buffering the urine sample for countercurrent analysis. The urine samples and rinse were collected for 10-min periods; the final volume of each sample was brought up to 10 ml by adding buffer.

A control solution of morphine-*N*[^{14}C]H_3 hydrochloride, 3–10 μc, in 100 ml of 0·85 or 0·45% sodium chloride containing *p*-aminohippuric acid (PAH; 0·125 μmole/kg/min), morphine sulfate (0·129 μmole/kg/min) and inulin (50 μg/kg/min) was infused into one saphenous vein at a rate of 0·388 ml min with a Harvard pump. Morphine-*N*[^{14}C]H_3 hydrochloride (17·6 and 57·0 mc/m-mole), hereafter designated as [^{14}C]-morphine, was obtained from Amersham/Searle. To test the inhibitors, SKF 525A, MPDC or SKF 2314 was added at varying doses to the control solution. In later experiments, tetraethyl-1-[^{14}C]ammonium bromide ([^{14}C]TEA; 3 mc/m-mole from New England Nuclear Corp.) was used in place of [^{14}C]morphine to assess the effect of SKF 525A and MPDC on the cationic organic base transport system.

The experimental protocol usually consisted of infusing the [^{14}C]morphine control solution into a saphenous vein while three to five 10-min urine collections were taken as controls. Then, the [^{14}C]morphine solution with one of the inhibitors was infused for 30–90 min (3–9 urine collection periods). In some experiments a single dose and in others progressively higher doses of the inhibitor were infused. In a few of the latter experiments, several periods of control infusion of [^{14}C]morphine without inhibitor were interspersed between periods with the inhibitor.

Total radioactivity in the urine samples was determined by plating 0·1–1·0 ml and counting on a Tracerlab low background system. In a few experiments, samples were counted on a Packard Tri-Carb scintillation spectrometer by placing 0·1 or 0·2 ml urine in 15 ml of scintillation fluid prepared with 5 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.[17]

In a number of experiments with morphine, the total radioactivity was fractionated by countercurrent distribution in order to determine the amount of the excreted radioactivity that was [^{14}C]morphine and [^{14}C]morphine-3-ethereal sulfate.[12,14,15] Two ml of the urine samples was adjusted to a pH of 8·4–8·6 by addition of concentrated ammonium hydroxide with a 10-μl Hamilton syringe. The countercurrent distribution analyses consisted of 2 ml of each phase of an $NaHCO_3$ (2 g/l.) buffer, pH 8·4–8·6, and chloroform (with 1 %, v/v, *N*-butanol) in a 9-tube (8-transfer) system as described previously. The chloroform phase was the mobile phase and was transferred manually with a syringe equipped with a long needle. One ml of each phase was plated and the radioactivity counted. In the urine samples, PAH was estimated by the method of Smith *et al.*;[18] inulin was determined by the method of Schreiner.[19]

The apparent tubular excretion fraction (ATEF) was calculated as a measure of morphine transport[12,14] by the formula proposed by Sperber[20] and modified by Lindahl and Sperber[21] where

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

EXC_I was the amount excreted from the infused side EXC_C was the amount excreted from the contralateral side, and *INF* was the amount infused during the period. ATEF values larger than 10 per cent have been taken as indicating active transport.[21,22]

A few experiments were done using pentobarbital-2-[^{14}C] (obtained from New England Nuclear Corp.) in place of [^{14}C]morphine. The pentobarbital (sp. act., 2·8 mc/m-mole) infusion solution was prepared as above for the morphine solutions. Samples were collected for 5-min periods and the volumes brought up to 5 ml with water. Total ^{14}C-radioactivity was measured as above. The ^{14}C-radioactivity was separated into parent compound and metabolite fraction by countercurrent distribution. Two ml urine was acidified with 1 ml of 20 % acetic acid in distilled water. The solvent system consisted of two phases of an equilibrated mixture of ethyl ether–glacial acetic acid–distilled water (10:1:10, by vol.). A 7-tube (6-transfer) distribution was performed using 3 ml of each phase. The mobile aqueous phase was transferred manually as above. Two-tenths ml of each phase was placed in 15 ml of scintillation fluid and the radioactivity counted.

RESULTS

Effect of SKF 525A and MPDC on inulin and PAH excretion. Since PAH was to be used as a marker substance for our transport studies, it was necessary to see whether SKF 525A and MPDC affected the transport of PAH. In Table 1 during control periods of infusion, the PAH ATEF was high and this ATEF was indicative of the active renal tubular transport of PAH. Infusion of either SKF 525A or MPDC led to a fall in this ATEF. Since PAH is known to be transported by the anionic organic acid transport system in the chicken, it was unlikely that this fall in ATEF was a direct effect on PAH transport. Both SKF 525A and MPDC are organic bases and would not be expected to affect the anionic system which transported PAH. It was more likely that the fall in PAH ATEF was caused by an opening of the venous bypass valve in the renal portal system because the per cent recovery of PAH during all periods was near 100 per cent. Even though the ATEF fell, the recovery did not fall. Further

TABLE 1. EFFECT OF SKF 525A AND MPDC ON INULIN AND PAH EXCRETION*

Chicken No.	Compound	Dose (mg/kg/min)	No. of 10-min periods	PAH		Inulin
				% Recovery	ATEF	Inf/Non-inf
44	Control†	0	3	101 ± 3	92 ± 1	0·96 ± 0·09
	SKF 525A	0·02	3	103 ± 3	76 ± 3	0·96 ± 0·05
	SKF 525A	0·1	3	98 ± 2	57 ± 3	1·00 ± 0·05
	SKF 525A	0·2	4	98 ± 2	46 ± 1	0·94 ± 0·05
45	Control†	0	3	98 ± 3	34 ± 1	0·99 ± 0·02
	MPDC	0·02	3	97 ± 2	26 ± 1	0·98 ± 0·03
	MPDC	0·1	3	96 ± 3	11 ± 1	0·99 ± 0·09
	MPDC	0·2	3	95 ± 3	5 ± 4	0·91 ± 0·05
	Control†	0	3	95 ± 3	19 ± 3	1·05 ± 0·03
46	Control†	0	3	98 ± 1	65 ± 1	0·93 ± 0·02
	MPDC	0·2	4	97 ± 2	41 ± 3	0·99 ± 0·03
	MPDC	0·02	4	98 ± 1	58 ± 3	1·02 ± 0·03
	MPDC	0·1	4	96 ± 2	49 ± 1	1·08 ± 0·04

* Values are given ± S.E. PAH = *p*-aminohippuric acid; ATEF = apparent tubular excretion fraction; MPDC = *N*-methyl-3-piperidyl-*N'*,*N'*-diphenylcarbamate.

† Control solution: PAH, 10 mg; inulin, 50 mg; NaCl, 450 mg in 100 ml of distilled water.

support for suggesting that the fall in PAH ATEF is not an effect on PAH transport but an effect on blood flow will be provided after results of the [^{14}C]TEA experiments are presented. Returning to Table 1, the inulin excretion data indicated that glomerular filtration of the two kidneys remained unchanged. Thus, these experiments suggested that PAH would still serve as a useful marker of the anionic transport system, even though SKF 525A and MPDC had effects on the venous valve.

Effect of SKF 525A, MPDC and SKF 2314 on morphine transport. Since [^{14}C]morphine, like PAH, is actively transported by the renal tubules of the chicken,[12] high [^{14}C]ATEF values were obtained as seen during the initial control infusion periods in Tables 2, 3 and 4. The effects of SKF 525A, MPDC and SKF 2314 were evaluated by infusing these agents along with the [^{14}C]morphine. At the smaller doses of SKF 525A, if the ATEF for PAH fell, a parallel fall in ATEF for ^{14}C from the morphine occurred. Thus, the ^{14}C/PAH ATEF ratio remained unchanged from control conditions. These findings meant that SKF 525A at small doses was not having any differential effect on morphine transport compared to PAH transport.

At larger doses of SKF 525A, the expected fall in PAH ATEF occurred due to opening of the venous valve. But now a more drastic effect on ^{14}C ATEF was seen. Thus at the high dose range of 0·32–2·25 mg/kg/min infusion rate of SKF 525A, a greater fall in ^{14}C ATEF than in PAH ATEF led to decreases in the ^{14}C/PAH ATEF ratio. These were interpreted to indicate that SKF 525A inhibited transport of [^{14}C]-morphine into the cell. The reason why such an effect could not be due to inhibition of metabolism of morphine is given in subsequent sections.

Once the SKF 525A infusion was terminated and the control morphine solution infused again, the ^{14}C ATEF returned toward control values, implying that the effect of this compound on morphine transport was reversible. The same can be said about the effect on the venous valve.

TABLE 2. EFFECT OF SKF 525A ON [^{14}C]MORPHINE AND PAH TRANSPORT*

Chicken No.	Compound	Dose (mg/kg/min)	No. of 10-min periods	Apparent tubular excretion fraction		
				^{14}C	PAH	^{14}C/PAH
34	Control†	0	3	42 ± 2	73 ± 3	0·54 ± 0·02
	SKF 525A	0·02	3	43 ± 1	78 ± 1	0·55 ± 0·02
	Control†	0	4	34 ± 4	72 ± 2	0·47 ± 0·05
	SKF 525A	0·04	4	21 ± 1	39 ± 3	0·55 ± 0·06
32	Control†	0	3	39 ± 2	72 ± 3	0·56 ± 0·07
	SKF 525A	0·1	3	19 ± 1	53 ± 5	0·37 ± 0·04
	Control†	0	5	31 ± 5	62 ± 8	0·49 ± 0·10
	SKF 525A	0·5	3	8 ± 6	31 ± 6	0·20 ± 0·09
38	Control†	0	3	35 ± 3	47 ± 2	0·81 ± 0·04
	SKF 525A	2·25	2	7 ± 5	26 ± 4	0·27 ± 0·10
	Control†	0	4	3 ± 1	10 ± 2	0·30 ± 0·02
40	Control†	0	3	33 ± 3	72 ± 1	0·47 ± 0·04
	SKF 525A	0·16	3	27 ± 1	60 ± 4	0·45 ± 0·01
	SKF 525A	0·32	3	22 ± 1	60 ± 1	0·37 ± 0·01
	SKF 525A	0·64	3	16 ± 1	56 ± 2	0·27 ± 0·02
96	Control†	0	5	31·9 ± 4·3	55·9 ± 6·3	0·57 ± 0·03
	SKF 525A	0·5	9	6·4 ± 2·3	31·1 ± 2·5	0·17 ± 0·06
98	Control†	0	5	11·5 ± 2·5	15·0 ± 4·0	0·94 ± 0·20
	SKF 525A	0·5	9	7·8 ± 0·6	27·3 ± 2·5	0·30 ± 0·02
100	Control†	0	5	29·9 ± 1·9	62·2 ± 4·8	0·48 ± 0·02
	SKF 525A	0·5	9	13·1 ± 1·3	57·4 ± 2·7	0·23 ± 0·02

* Values are given ± S.E. See Table 1 for abbreviations.
† Control solution as in Methods.

In Table 3, experiments performed with MPDC show results similar to those seen with SKF 525A. A fall in ^{14}C ATEF to below 10 and a fall in the ^{14}C/PAH ATEF ratio were found. Thus MPDC, like SKF 525A, inhibited renal tubular transport of [^{14}C]morphine. Also, a tendency to return to initial control values, seen in experiment 47 performed with the lower doses of MPDC, pointed to a reversible effect of MPDC on morphine transport and on opening of the valve.

The experiments in which SKF 2314 was infused are shown in Table 4. This compound appeared to affect the venous valve in a manner similar to SKF 525A and MPDC. However, the effects were generally not convincingly manifested on transport of morphine.

Effect of SKF 525A, MPDC and SKF 2314 on morphine metabolism. Table 5 summarizes the data of four experiments in which countercurrent distribution analyses were performed on the ^{14}C excreted in the urine by the infused and noninfused kidney. From previous work it is known that the excess of morphine ethereal sulfate (MES) found in the urine from the infused side compared to the noninfused side arises from the intracellular metabolism of morphine in the renal tubules of the infused kidney.[12,14] For example, 30,989 minus 9690 gives 21,299 counts/min excess during the control period in chicken 32; this excess represents intracellularly formed MES. If SKF 525A were to inhibit this intracellular metabolism, a decrease in this excess would be expected. Since less of the intracellular morphine would then be converted to MES, a

TABLE 3. EFFECT OF MPDC ON [^{14}C]MORPHINE AND PAH TRANSPORT*

Chicken No.	Compound	Dose (mg/kg/min)	No. of 10-min periods	Apparent tubular excretion fraction		
				^{14}C	PAH	^{14}C/PAH
30	Control†	0·0	3	31 ± 2	75 ± 8	0·38 ± 0·03
	MPDC	0·5	4	7 ± 5	33 ± 5	0·19 ± 0·05
	Control†	0·0	3	4 ± 2	30 ± 1	0·13 ± 0·05
31	Control†	0	3	30 ± 5	70 ± 10	0·49 ± 0·02
	MPDC	0·5	3	10 ± 2	50 ± 3	0·20 ± 0·02
	Control†	0	3	4 ± 1	53 ± 2	0·07 ± 0·01
47	Control†	0	3	49 ± 1	59 ± 1	0·83 ± 0·02
	MPDC	0·05	4	32 ± 4	47 ± 2	0·70 ± 0·02
	MPDC	0·1	4	21 ± 1	49 ± 1	0·43 ± 0·06
	MPDC	0·2	4	11 ± 3	30 ± 5	0·35 ± 0·06
	Control†	0	3	26 ± 4	45 ± 4	0·58 ± 0·02
102	Control†	0	5	33·9 ± 1·9	26·0 ± 3·0	1·35 ± 0·10
	MPDC	0·5	9	5·5 ± 2·1	17·6 ± 5·6	0·34 ± 0·10
104	Control†	0	5	31·4 ± 3·0	30·1 ± 2·8	1·04 ± 0·04
	MPDC	0·5	9	6·7 ± 2·3	14·0 ± 2·2	0·17 ± 0·10
106	Control†	0	5	42·6 ± 4·6	66·0 ± 6·4	0·64 ± 0·02
	MPDC	0·5	9	8·8 ± 0·8	31·6 ± 3·6	0·29 ± 0·01

* Values are given ± S.E. See Table 1 for abbreviations.
† Control solution as in Methods.

TABLE 4. EFFECT OF SKF 2314 ON [^{14}C]MORPHINE AND PAH TRANSPORT*

Chicken No.	Compound	Dose (mg/kg/min)	No. of 10-min period	Apparent tubular excretion fraction		
				^{14}C	PAH	^{14}C/PAH
41	Control†	0	3	51 ± 1	81 ± 2	0·63 ± 0·03
	SKF 2314	0·015	3	32 ± 3	51 ± 2	0·64 ± 0·04
	SKF 2314	0·03	3	27 ± 1	45 ± 1	0·60 ± 0·01
	SKF 2314	0·06	3	34 ± 1	57 ± 2	0·60 ± 0·02
42	Control†	0	4	28 ± 2	43 ± 3	0·64 ± 0·01
	SKF 2314	0·016	3	15 ± 2	17 ± 2	0·87 ± 0·07
	SKF 2314	0·08	3	13 ± 1	20 ± 1	0·70 ± 0·02
	SKF 2314	0·16	3	9 ± 2	12 ± 2	0·74 ± 0·03
43	Control†	0	3	26 ± 2	45 ± 3	0·56 ± 0·01
	SKF 2314	0·12	4	20 ± 3	38 ± 4	0·51 ± 0·02
	Control†	0	16	29 ± 2	45 ± 3	0·61 ± 0·03
	SKF 2314	0·48	4	26 ± 3	54 ± 6	0·48 ± 0·02

* Values are given ± S.E. See Table 1 for abbreviations.
† Control solutions as in Methods.

relative rise in excess of morphine by the ipsilateral kidney should result. The data do not support such a mechanism. Examining the data during infusion of 0·1 mg/kg/min of SKF 525A shows that now the excess in MES is 26,393 minus 15,383 or 11,010 counts/min. Therefore, half as much MES (11,010 vs. 21,299 counts/min) is now being excreted by this kidney. But, note that total ^{14}C ATEF also went down during this period (as discussed earlier) so that less morphine was available within the cell for metabolism to take place. In fact, all the drop in MES can be accounted for by this latter mechanism, since the per cent of the total ^{14}C excreted during this time as MES was 57 per cent compared to 52 per cent in the absence of SKF 525A. The proportion of the metabolite MES did not go down. Therefore, the only conclusion possible under these circumstances is that SKF 525A inhibited transport of morphine into the cell, but did not inhibit its metabolism.

From a dose response point of view, the experiment on chicken 34 showed that little change occurred in the relative amount of morphine and metabolite excreted with the infusion of 0·02 mg/kg/min of SKF 525A. When the dose of SKF 525A was doubled, a decrease in absolute amount of both morphine and MES was found on the infused side. This inhibitory effect was more marked in the other experiments performed with higher doses of SKF 525A. These decreases went hand in hand with the decrease in ^{14}C ATEF. Also, the percentage of ^{14}C excreted as ^{14}C MES did not fall. Therefore, this metabolism was not affected by SKF 525A. Yet transport of morphine had been inhibited as indicated earlier.

The same conclusions were derived about the effect of MPDC as indicated by the results in Table 6. The simultaneous fall of [^{14}C]morphine and ^{14}C MES meant that transport of morphine rather than metabolism was being affected by MPDC. A further point of explanation is necessary in this case for results which are not evident in Table 6. Countercurrent analyses of urine from chickens treated with MPDC showed a shift of the peak for free morphine from tube 3 to tube 8. Since addition of MPDC to [^{14}C]morphine-containing urine produced the same effect, the [^{14}C]morphine was calculated from the fraction of ^{14}C found in tubes 5–8 in the countercurrent system. This unusual situation was thoroughly investigated.[23]

The experiments performed with SKF 2314 (Table 7) show no discernible change in the amount of radioactivity excreted as morphine and MES at the different doses of SKF 2314 used. The same was true in relation to the per cent of each component in each sample. It appears that doses were insufficient to show effects either on transport or metabolism.

Effect of SKF 525A and MPDC on transport of [^{14}C]TEA. Since the results suggested that both SKF 525A and MPDC inhibited transport of morphine, these two compounds were tested against [^{14}C]TEA, a compound which is transported by the cationic organic base system and which is not metabolized to any large extent.[24] In the five experiments in Table 8 with SKF 525A, the ^{14}C/PAH ratio was consistently lowered by the SKF 525A. Similarly, MPDC lowered the ^{14}C/PAH ratio in all three experiments. Thus, these results unequivocally demonstrate that SKF 525A and MPDC differentially blocked the cationic system for [^{14}C]TEA transport as compared to the PAH transport.

Effect of SKF 525A and MPDC on pentobarbital metabolism. The results of experiments in which the effect of SKF 525A and MPDC on pentobarbital-2-^{14}C were assessed are shown in Table 9. About equal amounts of ^{14}C were excreted from the

TABLE 5. COUNTERCURRENT DISTRIBUTION ANALYSES OF RADIOACTIVITY FOUND IN URINE AFTER INFUSION OF [C^{14}]-MORPHINE AND SKF 525A

Chicken No.	Compound	Dose (mg/kg/min)	No. of 10-min periods	EXC_I* (counts/mmin/period)		EXC_C* (counts/min/period)		EXC_I (%)		EXC_C (%)	
				M†	MES†	M	MES	M	MES	M	MES
32	Control‡	0	3	28,685	30,989	7090	9690	48	52	42	58
	SKF 525A	0·1	3	19,280	26,393	8595	15,383	43	57	36	64
	Control‡	0	5	29,712	32,914	10,342	18,883	47	53	35	65
	SKF 525A	0·5	3	15,330	21,976	9540	20,053	41	59	32	68
34	Control‡	0	3	20,770	24,970	3900	7500	46	54	35	65
	SKF 525A	0·02	3	22,790	29,110	5240	10,860	44	56	33	67
	Control‡	0	4	23,680	27,000	6950	11,590	48	52	38	62
	SKF 525A	0·04	4	18,880	25,310	8460	17,640	43	57	32	68
38	Control‡	0	3	35,340	34,990	8950	16,290	51	49	36	64
	SKF 525A	2·25	2	8440	17,310	5590	7900	32	68	24	76
	Control‡	0	4	12,390	25,200	10,310	24,940	31	69	23	77
40	Control‡	0	3	18,333	19,033	4466	7866	49	51	36	64
	SKF 525A	0·16	3	17,233	19,466	5500	11,600	47	53	32	68
	SKF 525A	0·32	3	16,000	19,433	6300	14,533	47	53	30	70
	SKF 525A	0·64	3	13,000	16,200	7633	16,300	44	56	32	68

* Designations as in Methods.
† M = [^{14}C]morphine; MES = morphine-3-ethereal sulfate.
‡ Control solution as in Methods.

TABLE 6. COUNTERCURRENT DISTRIBUTION ANALYSES OF RADIOACTIVITY FOUND IN URINE AFTER INFUSION OF [^{14}C]MORPHINE AND MPDC

Chicken No.	Compound	Dose (mg/kg/min)	No. of 10-min periods	EXC_I* (counts/min/period)		EXC_C* (counts/min/period)		EXC_I (%)		EXC_C (%)	
				M†	MES†	M	MES	M	MES	M	MES
30	Control‡	0	3	30,000	38,890	8290	11,722	43	57	45	55
	MPDC	0·5	4	16,050	22,925	9122	17,837	41	59	32	68
	Control‡	0	3	16,956	25,288	12,326	24,156	40	60	33	67
31	Control‡	0	3	33,050	17,050	16,000	10,100	66	34	63	38
	MPDC	0·5	3	22,080	18,390	17,776	14,810	54	46	54	46
	Control‡	0	3	18,130	16,480	17,080	14,830	56	44	53	47
47	Control‡	0	3	18,966	12,100	4900	5366	60	40	48	52
	MPDC	0·05	4	15,400	11,500	4800	6625	57	43	42	58
	MPDC	0·1	4	11,300	13,150	5925	8150	46	54	42	58
	MPDC	0·2	4	7725	14,000	5500	10,500	35	65	34	66
	Control‡	0	3	13,435	16,000	5933	10,700	46	54	35	65

* Designations as in Methods.
† M = [^{14}C]morphine; MES = morphine-3-ethereal sulfate.
‡ Control solutions as in Methods.

TABLE 7. COUNTERCURRENT DISTRIBUTION ANALYSES OF RADIOACTIVITY FOUND IN URINE AFTER INFUSION OF [^{14}C]MORPHINE AND SKF 2314

Chicken No.	Compound	Dose (mg/kg/min)	No. of 10-min periods	EXC_I* (counts/min/period)		EXC_C* (counts/min/period)		EXC_I (%)		EXC_C (%)	
				M†	MES†	M	MES	M	MES	M	MES
41	Control‡	0	3	41,633	32,133	8200	9644	57	43	46	54
	SKF 2314	0·015	3	34,633	28,100	11,800	15,233	55	45	44	56
	SKF 2314	0·03	3	31,166	26,866	11,333	16,766	55	45	41	59
	SKF 2314	0·06	3	31,733	31,033	10,633	14,666	51	49	41	59
42	Control‡	0	4	37,125	28,050	12,250	15,250	57	43	44	56
	SKF 2314	0·016	3	29,466	23,066	14,133	18,433	56	44	43	57
	SKF 2314	0·08	3	30,100	28,000	16,000	22,500	52	48	42	58
	SKF 2314	0·16	3	29,700	26,830	18,700	25,933	53	47	42	58
43	Control‡	0	3	29,866	21,566	8966	14,600	59	41	38	62
	SKF 2314	0·12	4	29,650	19,525	11,350	16,325	59	41	41	59
	Control‡	0	16	37,568	24,643	12,363	16,675	60	40	42	58
	SKF 2314	0·48	4	46,625	29,325	15,250	20,450	61	39	42	58

* Designations as in Methods.
† M = [^{14}C]morphine; MES = morphine-3-ethereal sulfate.
‡ Control solution as in Methods.

TABLE 8. EFFECT OF SKF 525A AND MPDC ON [^{14}C]TEA AND PAH TRANSPORT*

Chicken No.	Compound	Dose (mg/kg/min)	No. of 10-min periods	Apparent tubular excertion fraction		
				^{14}C	PAH	^{14}C/PAH
58	Control	0	4	24·1 ± 3·5	40·3 ± 2·1	0·60 ± 0·08
	SKF 525A	0·2	11	17·4 ± 4·5	48·1 ± 1·8	0·38 ± 0·11
60	Control	0	5	36·7 ± 5·4	41·6 ± 4·9	0·87 ± 0·05
	SKF 525A	0·4	9	5·2 ± 13·2	46·1 ± 3·1	0·16 ± 0·28
82	Control	0	6	62·0 ± 3·4	96·5 ± 5·5	0·66 ± 0·06
	SKF 525A	0·5	9	40·4 ± 2·4	100·9 ± 5·6	0·40 ± 0·02
86	Control	0	6	43·3 ± 1·7	45·6 ± 2·3	0·96 ± 0·04
	SKF 525A	0·5	9	15·3 ± 2·0	34·7 ± 2·3	0·44 ± 0·04
88	Control	0	6	69·2 ± 5·6	66·8 ± 0·28	1·05 ± 0·10
	SKF 525A	0·5	8	13·9 ± 5·4	40·2 ± 6·2	0·29 ± 0·10
90	Control	0	6	61·9 ± 2·2	66·2 ± 2·6	0·94 ± 0·04
	MPDC	0·5	9	−1·3 ± 4·2	33·1 ± 2·6	−0·08 ± 0·10
92	Control	0	6	74·5 ± 3·7	56·9 ± 4·5	1·33 ± 0·07
	MPDC	0·5	9	−2·7 ± 3·9	41·5 ± 3·8	−0·11 ± 0·07
94	Control	0	6	68·2 ± 3·2	67·9 ± 3·2	1·01 ± 0·07
	MPDC	0·5	9	−6·5 ± 4·0	47·3 ± 4·3	−0·14 ± 0·11

* [^{14}C]TEA = tetraethyl-1-^{14}C ammonium bromide. See Table 1 for other abbreviations..

two sides, even though the ^{14}C-pentobarbital was infused on one side. Therefore, no active transport for ^{14}C-pentobarbital occurred and the ATEF was near zero. Table 9 also shows that, after infusion of SKF 525A, there was a marked decrease in the amount of total ^{14}C as well as ^{14}C-metabolite appearing in the urine of both the infused and noninfused sides; a decreased recovery in total radioactivity occurred. The result of the countercurrent analyses (expressed as a ratio of the pentobarbital/metabolite) showed that little pentobarbital (5–15%) was excreted unchanged and most of the radioactivity found in the urine (85–95%) was metabolite. Thus, the decrease in total ^{14}C excreted after SKF 525A infusion was due mainly to a decrease in the amount of ^{14}C-metabolite. This change is indicated by the increase in the pentobarbital/metabolite ratio with SKF 525A infusion while the ^{14}C excretion fell markedly. This effect on metabolite excretion was still present 90 min after discontinuing the SKF 525A infusion.

The experiment with MPDC showed the same effects as with SKF 525A. The main difference between these drugs was that MPDC had a less persistent effect than SKF 525A, since after 90 min the amount of total ^{14}C appearing in the urine was back to control values.

DISCUSSION

Both SKF 525A and MPDC opened the venous valve to cause shunting of the blood away from the ipsilateral renal peritubular capillary system into the systemic circulation. This shunting resulted in a fall in PAH ATEF; a proportional fall in ^{14}C ATEF for morphine must be attributed to this shunting. That the fall in PAH ATEF

TABEL 9. EFFECT OF SKF 525A AND MPDC ON PENTOBARBITAL-2-^{14}C METABOLISM

Chicken No.	Compound*	No. of 5-min periods	EXC_I† (counts/min/period)	EXC_C† (counts/min/period)	% Recovered	Pentobarbital/Metabolite‡ EXC_I	Pentobarbital/Metabolite‡ EXC_C
36	Control§	6	44,133 ± 4960	42,433 ± 2978	44 ± 3	0·08	0·05
	SKF 525A‖	6	30,733 ± 6782	30,666 ± 5075	30 ± 5	0·15	0·06
	Control§	6	26,233 ± 463	24,633 ± 578	25 ± 1	0·27	0·15
49	Control§	6	32,070 ± 1800	31,045 ± 1680	50 ± 2	0·18	0·14
	MPDC	6	18,570 ± 860	16,330 ± 800	28 ± 1	0·11	0·11
	Control§	3	21,750 ± 1620	23,200 ± 1250	36 ± 2	0·10	0·09
	Control§	3	37,780 ± 1450	36,130 ± 1350	59 ± 2	0·09	0·08
50	Control§	6	32,750 ± 3180	30,850 ± 2780	51 ± 3	0·24	0·19
	SKF 525A¶	6	13,400 ± 1380	14,310 ± 1280	22 ± 2	0·63	0·30
	Control§	6	9570 ± 800	10,050 ± 800	16 ± 1	0·34	0·19
	Control§	1	12,800	12,850	20	0·20	0·18

* After every change and before collections, 30 min were allowed.
† Designations as in Methods.
‡ The standard error ranged from 0·01 to 0·05.
§ PAH 10 mg/100 ml of 0·5% saline with pentobarbital-2-^{14}C (14 μc). The apparent tubular excretion factor for PAH during control periods fluctuated from 60 to 82.
‖ Control plus 0·1 mg/kg/min.
¶ Control plus 0·5 mg/kg/min.

was largely an effect on the valve is indicated by the following evidence. Sperber[20] demonstrated that PAH was extracted completely on its initial pass through the peritubular capillary network and any PAH that appeared in the systemic circulation had bypassed the tubular system. Thus, ATEF for PAH was considered to reflect the patency of the valve, a high ATEF indicating a closed valve and a low ATEF indicating an open valve. The validity of this view has been supported by Rennick and Gandia,[25] who showed that the valve was under cholinergic control for contraction and under adrenergic control for relaxation. Atropine decreased the excess PAH excreted by the infused kidney through an effect on the valve. Since SKF 525A and MPDC have been shown to possess atropine-like activity,[3,26,27] such activity would explain the effect of these drugs on the venous valve in the present experiments. Since the total recovery of PAH during our experiment did not change under the influence of these drugs, it would be unlikely that tubular transport of PAH was affected. These arguments are consistent with the results of the [^{14}C]TEA experiment. Since both PAH and TEA are actively transported, opening of the venous valve alone should have caused a parallel fall in the ATEF of both PAH and [^{14}C]TEA. However, a greater fall occurred in the [^{14}C]TEA than in the PAH ATEF. Although these results do not completely rule out an effect of SKF 525A and MPDC on PAH transport, the obviously greater sensitivity of the cationic transport system to SKF 525A and MPDC is evident. Even though opening of the valve complicated the results, the interpretation of the effect of these drugs on morphine transport was clear.

The data indicated that SKF 525A and MPDC inhibited transport of morphine in the chicken kidney. This interpretation was deduced from the decrease in the ^{14}C ATEF for ^{14}C-morphine infusion and also from the decrease in the ATEF ratio of ^{14}C/PAH. The fall in the latter values can be accepted as a preliminary indication of blockade in transport of morphine. The general concept of using the differential fall in the ATEF of a compound relative to a marker has been widely applied by others.[20,28–31] The countercurrent analyses showed a marked decrease in the excretion of the morphine fraction by the ipsilateral kidney. The fraction corresponding to the morphine-3-ethereal sulfate was decreased proportionately. In these and in additional cases where total ^{14}C excretion was measured, a dramatic drop in total radioactivity occurred. In the remaining discussion, the arguments for and evidence supporting the site of action of SKF 525A and MPDC on the transport of morphine will be presented. Arguments against other sites of actions such as on metabolism of morphine and transport of the metabolite will be developed.

At this point, the reader should refer to the work by May *et al.*[12] and Watrous *et al.*[14,15] regarding the mechanism of renal tubular transport of morphine and the intracellular metabolism of morphine to MES. Since SKF 525A and MPDC do not inhibit the transport of MES (administered by infusion), any fall in ^{14}C-MES excretion after [^{14}C]morphine administration and SKF 525A and MPDC infusion must be attributed to lack of access of morphine into the cell. That is, we know that if there is any MES formed within the cell, its translocation from the cell to the tubular lumen should still be unimpeded (since MES transport is unaffected). An alternative would be to argue that inhibition of metabolism of morphine by SKF 525A and MPDC within the cell not only leads to a decrease in formation of MES (which results in decreased MES excretion) but also somehow results in a decrease in [^{14}C]morphine transport. These actions would then result in decreased ^{14}C ATEF and decreased

excretion of [^{14}C]morphine and [^{14}C]MES. This alternate argument does not fit the expectations. From the work of Watrous and Fujimoto,[15] it is established that inhibition of metabolism of morphine to MES is not in itself sufficient to inhibit morphine transport. In their experiment, inhibition of metabolism of morphine to MES (by catechol) led to an increase in excretion of unchanged [^{14}C]morphine with the total [^{14}C]ATEF not being decreased. Since catechol did not block transport of MES nor block access of morphine into the cell, inhibition of the metabolism of morphine to MES resulted in the excretion of the excess morphine within the cell as unchanged morphine; the total amount of [^{14}C]morphine transported across the peritubular membrane of the renal tubular cell was still the same. With these results in mind, one would expect in the present experiment that if SKF 525A and MPDC were inhibiting metabolism of morphine to MES, the results should be identical to those in which catechol was used. The results are obviously different; therefore the alternate hypothesis is incompatible with the results. SKF 525A and MPDC did not inhibit metabolism.

More direct evidence is available to support the hypothesis that SKF 525A and MPDC are acting on the cationic transport system. [^{14}C]TEA transport was differentially reduced over that of PAH transport as shown in Table 8. Since TEA is metabolized little if at all, differential reduction in its ATEF over that of PAH must be an effect on transport of TEA. In this sense, the effect of SKF 525A and MPDC in blocking transport of morphine would be due to competition as organic bases. Other organic bases such as mepiperphenidol and cyanine 863 block morphine transport.[10,14] Since it was surmised that one probable location of the base transport system was on the peritubular side of the renal tubular cell,[17] competition between SKF 525A and MPDC for morphine transport might be occurring at a peritubular site. Such a site of action would be highly conformable with our present results. In this context, it is interesting that one of the earliest suggestions for a site of action of SKF 525A in another system (hepatic microsomes) was on a membrane.[4] More recently, Lee *et al.*[32] showed that SKF 525A had several effects on red blood cell membranes and Gaurez-Kurtz and Bianchi[33] noted the similarity in action between SKF 525A and local anesthetics. Permeability may be affected in a more general sense as shown by Marchand *et al.*[34] on the gastrointestinal absorption of CCl_4; the surface active properties of SKF 525A have been studied by Florence.[35] It is indeed intriguing that Henderson and Dewaide[36] found that inhibition by SKF 525A of glucuronidation of *p*-nitrophenol occurred in the intact hepatocyte but not in homogenates. They suggested that for UDP-glucuronidation, SKF 525A produced a nonspecific change in the properties of the membranes. One wonders how much of the effects of SKF 525A are explainable on the basis of an action of a translocation process associated with a membrane rather than direct inhibition of an enzyme.

Since SKF 2314 was reported to be an enzyme inhibitor,[7] it was logical to test this compound. Being an acid, it should not compete with morphine for transport, since morphine is transported only by the base system.[12–14] SKF 2314 had no effect on transport and no effect on metabolism of morphine to MES. These data were not helpful in clarifying the present situation, but were consistent with our proposed mechanism.

The insensitivity of morphine metabolism to inhibition by SKF 525A, 2314 and MPDC may arise from the fact that the enzyme responsible for this conjugation

process, formation of ethereal sulfates, are located probably in the soluble fraction of the cell[37,38] rather than in the endoplasmic reticulum. The main inhibitory action of SKF 525A, MPDC and SKF 2314 appears to be on microsomal enzymes systems,[28] and SKF 525A can act as alternative substrate for drug-metabolizing microsomal enzymes.

In the experiments with pentobarbital-^{14}C, metabolism was blocked by SKF 525A and MPDC. When pentobarbital-^{14}C was infused into one saphenous vein, the ATEF for ^{14}C excretion was near zero. The acidic pH (4·5–6·5) of chicken urine favors the reabsorption of pentobarbital (pK_a 8·0) and excretion of free ^{14}C-pentobarbital was low in the urine. Between 85 and 95 per cent of the excreted ^{14}C-radioactivity was metabolite, with from 5 to 15 per cent being pentobarbital-^{14}C by countercurrent analyses. The pentobarbital that may diffuse into the renal cell was not metabolized very rapidly. Based on a nonionic mechanism, one may except an excess of pentobarbital-^{14}C to be present in the renal tubular cells on the ipsilateral side. Yet no excess of metabolite was excreted from the infused side compared to the noninfused side. Thus, metabolism of the major portion of pentobarbital occurred in organs other than the kidney. This metabolism of pentobarbital by other organs was blocked by SKF 525A and MPDC. The block in metabolism brought about a decrease in the amount of metabolite being excreted from both sides. Increase in the pentobarbital fraction did not occur. The results were compatible with the known effects of SKF 525A and MPDC barbiturate metabolism in the liver.[10,11]

The duration of action of SKF 525A and MPDC on some of the described effects is of interest. The blocking action of SKF 525A and MPDC on morphine transport was short; the block decreased after stopping their infusion. In contrast, the effect of these drugs on inhibition of pentobarbital metabolite excretion was relatively long lasting. These findings would be compatible with two different inhibitory sites of action for SKF 525A. One was blockade of transport as exemplified by morphine, and the other inhibition of metabolism as exemplified by pentobarbital.

Finally, others have demonstrated an effect of SKF 525A on the kidney. Marshall and Williamson[39] and Hook and Williamson[40] demonstrated a natriuretic effect of SKF 525A in the dog after intra-arterial injection; they reported that the duration of this effect was very brief.

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