TRANSPORT OF PAPAVERINE IN RAT KIDNEY CORTICAL SLICES

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(Received April 16, 1984)

Previous results (*J. Pharm. Dyn.*, 7, 342, 1984) from this laboratory indicated that papaverine, a classical phosphodiesterase inhibitor, inhibited the transport of organic anions such as *p*-aminohippurate (PAH) and urate in rat kidney cortical slices. The transport of papaverine itself, an organic cationic drug, in the kidney is not yet understood. The purpose of this study was to examine the interaction of papaverine with incubated slices and basolateral membrane vesicles prepared from rat kidney cortex, in terms of the possibility of the intracellular action of papaverine in kidney cells. Papaverine was taken up against a concentration gradient by kidney cortical slices and by liver slices. The uptake of papaverine by the former slices was depressed by hypothermia and anoxia with metabolic inhibitors, but that of the drug by the latter slices was not affected by hypothermic condition. Tetraethylammonium (TEA) as a prototype for organic cationic drugs did not depress papaverine transport. TEA also had no effect on the inhibitory effect of papaverine on PAH accumulation in kidney cortical slices. Papaverine, however, inhibited TEA accumulation prominently. Kinetic studies using the slices indicated that papaverine increased the K_m for TEA accumulation and decreased V_{max} . Then, papaverine inhibition was a "mixed type". TEA uptake by basolateral membrane vesicles was markedly inhibited by papaverine, but PAH uptake by the vesicles was not affected by the drug.

The present results indicate that papaverine may be at least partly transported by the same system which handles TEA, but some aspect of the transport system for papaverine may be qualitatively different from that for TEA. Additional studies are required, however, to unequivocally establish the relationships between papaverine and TEA renal transport mechanisms.

Keywords — papaverine; *p*-aminohippurate transport; tetraethylammonium transport; kidney cortical slice; basolateral membrane vesicle

Although phosphodiesterases from many different tissues were inhibited by papaverine,^{1, 2)} the pharmacological effects of papaverine on various mammalian tissues are more limited than those of the other classical phosphodiesterase inhibitor, theophylline. Pösch and Kukovetz explain that theophylline may be able to penetrate the cellular membrane of many tissues more easily than papaverine, and then the latter has no access to the enzyme.¹⁾

Previous *in vitro* observations on transport of organic anions in rat kidney cortical slices demonstrated that papaverine inhibited the ac-

cumulation of *p*-aminohippurate (PAH) and urate in the slices.³⁾ If the inhibitory effect of papaverine is due to an intracellular action of the drug, papaverine could be taken up by kidney cells. It is well-known that tetraethylammonium (TEA), as a representative of organic cationic drugs, is accumulated in kidney slices *via* the transport system for organic cations in renal cellular membranes.^{4, 5)} Papaverine is also an organic cation. Previously no study has been made of papaverine transport in kidney, nor has it been determined to what degree papaverine affects TEA transport.

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The studies reported here were designed to characterize papaverine transport in rat kidney cortex *in vitro*.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 200–300 g and fed with a standard diet *ad libitum* were used in all experiments. Rat kidney cortical and liver slices (about 0.3–0.5 mm in thickness) prepared with a razor blade were placed in a chilled medium containing 134 mM NaCl, 5.9 mM KCl, 1.5 mM CaCl₂, 11.5 mM glucose and 5.8 mM Hepes (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid) buffer, pH 7.4. For the experiment of PAH accumulation in the slices, the slices were incubated at 37°C with a gas phase of 100% oxygen under a fully aerobic condition in 10 ml of an incubation medium; its composition was identical to the medium described above, except that it additionally contained 0.074 mM PAH and 1% inulin which was added to estimate the extracellular space of the slices. Incubation times were 30 min for the experiment of PAH accumulation, because the steady state accumulation was examined under the condition of 30 min-incubation.³⁾ For the measurement of papaverine accumulation the incubation medium contained 0.05 mM papaverine instead of PAH, and for that of TEA accumulation the incubation medium did 0.1 mM TEA plus ¹⁴C-TEA (5 nCi/ml) and methoxy-³H-inulin (100 nCi/ml) instead of PAH and inulin. The amounts of PAH, papaverine and TEA in the slices were determined on the basis of the measurement of intracellular water which was defined as wet weight of the slices minus fluid weight of the extracellular space minus dry weight after drying the slice at 100°C overnight. The concentrations of PAH and inulin in the slices and medium were determined spectrophotometrically as already described.⁶⁾ Papaverine in the slices and medium was analysed by the method of Axelrod *et al.*⁷⁾

Basolateral membranes were prepared from kidney cortex by a slight modification of the

method of Inui *et al.*⁸⁾ using a self-orienting Percoll gradient. Briefly, the supernatant and fluffy layer obtained by differential centrifugation of the homogenate in 250 mM sucrose, 1 mM ethylenediaminetetraacetate and 10 mM Tris-HCl (pH 7.5) at 2400 × *g* for 15 min were centrifuged at 20500 × *g* for 20 min. The resultant fluffy layer and pellet were suspended in above sucrose buffer and then recentrifuged at 20500 × *g* for 20 min. The fluffy layer yielded was resuspended and subjected to centrifugation at 40000 × *g* for 30 min on a Percoll gradient which consisted of 13.5% Percoll concentration. The basolateral membrane formed a band in the Percoll gradient which was located just above the Pharmacia blue marker bead (density = 1.037 g/ml) in a centrifuge tube. The membrane preparations were evaluated by measuring the specific activity of enzyme markers. The specific activity of (Na⁺ + K⁺)-ATPase, a basolateral membrane marker, was enriched about 15-fold of the activity of the crude homogenate. By contrast, the enrichment factor for alkaline phosphatase, a brush-border membrane maker, was only 1.3. For the measurement of TEA of PAH uptake, 0.02 ml of the basolateral membrane vesicle suspension was added to 0.1 ml of incubation medium. Its composition was 100 mM mannitol, 100 mM NaCl, 20 mM Tris-Hepes buffer, pH 7.5, and 0.454 mM ¹⁴C-TEA (2 μCi/ml) or 0.027 mM ³H-PAH (10 μCi/ml), respectively. In our preliminary experiments, the initial rates of PAH and TEA uptake were maintained at least for 15 s at 37°C. Therefore, the membrane vesicles were incubated for 15 s in this study. After incubation at 37°C the uptake of TEA or PAH was terminated by dilution of the reactants with 1 ml of ice-cold solution being identical to the incubation medium with the exception that ¹⁴C-TEA or ³H-PAH was omitted and by immediate filtration through 0.45-μm membrane filters (Sartorius SM 11306) under vacuum. Then, the filter was washed with 3 ml of the ice-cold solution and dissolved in Methyl Cellosolve. Protein was determined by the method of Lowry *et al.*⁹⁾

Radioactivities of ^3H and ^{14}C in the slices, medium and the membrane vesicles were measured in a liquid scintillation spectrometer

FIG. 1. *Effect of TEA on the Inhibition of PAH Accumulation by Papaverine*

Kidney cortical slices were incubated at 37°C for 30 min in the medium containing the indicated concentrations of papaverine and TEA. Each column indicates the mean of four experiments. A vertical line indicates the S.E. of the mean.

a) Significantly different from control at $p < 0.001$.

b) Significantly different from control at $p < 0.001$, but not significantly different from papaverine.

(Packard Tri-Carb 3255) as described previously.¹⁰⁾

The slice (S)-to-medium concentration (M) ratios (S/M) of PAH, papaverine and TEA were calculated.

[1- ^{14}C] Tetraethylammonium bromide and methoxy- ^3H -inulin were obtained from New England Nuclear Corp., Boston, Mass. *p*-Amino-2- ^3H -hippurate was purchased from Radiochemical Centre, Amersham, England. The values are expressed as mean $\pm$ S.E. Statistical analyses were performed by Student's *t* test. Regression analysis was performed by the method of least squares.

RESULTS

As is seen in Fig. 1, PAH accumulation was remarkably inhibited by papaverine and the results were similar to those obtained previously.³⁾ The ability of TEA to antagonize the inhibitory effect of papaverine was not observed by comparing the degree of the depression of PAH accumulation at 0.03 mM papaverine with or without 10 mM TEA. PAH accumulation was not affected by 10 mM TEA itself in the absence of papaverine (data not shown).

We studied whether papaverine, an organic cation, could be taken up by kidney cortical slices *via* the same transport system as that for TEA, as a prototype for organic cationic drugs,

TABLE I. *Accumulation of Papaverine in Kidney Cortical Slices Incubated under a Variety of Conditions*

	Papaverine accumulation (S/M)	Statistical significance of differences
Control	10.30 ± 0.51	
Hypothermic (0°C)	5.68 ± 0.19	$p < 0.005$
DNP + IAA + anaerobic (N_2)	7.52 ± 0.39	$p < 0.005$
TEA 10 mM	10.14 ± 0.35	Not significant

Kidney cortical slices were incubated at 37°C for 30 min in the medium containing 0.05 mM papaverine under indicated conditions.

The results represent the mean $\pm$ S.E. of four experiments.

DNP: 2,4-dinitrophenol (0.1 mM).

IAA: iodoacetate (0.1 mM).

TABLE II. Incubation of Liver Slices with Papaverine at 37 or 0°C

	Papaverine in slices (S/M)	Statistical significance of difference
Control (37°C)	6.47 ± 0.33	Not significant
Hypothermic (0°C)	5.95 ± 0.29	

Liver slices were incubated at 37 or 0°C for 30 min in the medium containing 0.05 mM papaverine. The results represent the mean ± S.E. of four experiments.

in order to elucidate the intracellular action of papaverine. Table I summarizes the data on papaverine accumulation and the effect of TEA on the accumulation. Papaverine was prominently taken up by kidney cortical slices and the S/M for the drug was 10.30 ± 0.51 . Papaverine accumulation was significantly depressed by hypothermia and anoxia with metabolic inhibitors. The response of TEA to papaverine accumulation was not observed at all. Liver slices also accumulated papaverine (Table II). However, the accumulation of the drug in liver slices was not affected by hypothermia. Accumulation of papaverine in these slices to S/M greater than 1.0 under the hypothermic and anoxic conditions may be due to passive cellular accumulation and binding to extracellular or membrane sites.

The next group of experiments was designed to show the effect of papaverine on the accumulation of TEA in the slices. Figure 2 represents the results of a typical experiment, in which a S/M ratio of TEA of about 16 was attained after 20 min-incubation in a medium containing 0.1 mM TEA. TEA accumulation was inhibited by papaverine at a concentration of 0.05 mM which depressed PAH accumulation. Figure 3 shows a experiment that was analyzed *via* the Lineweaver-Burke plot for kinetic characteristics. The tissue concentration of TEA over a 10-min period is double-reciprocally plotted as a function of medium TEA in the presence of absence of papaverine. It can be seen that both curves were rectilinear and did not intersect one another on the ordinate and the abscissa. Then, we have calculated the kinetic parameters for TEA transport. Table III gives the mean values of

FIG. 2. Effect of Papaverine on Accumulation of TEA in Kidney Cortical Slices

Kidney cortical slices were incubated at 37°C in the presence (●) or absence (○) of 0.05 mM papaverine. Each point is the mean of two experiments.

$V_{\max}$ and K_m for the medium with or without papaverine (0.05 mM). Papaverine increased the K_m for TEA accumulation and decreased $V_{\max}$. These data revealed that papaverine was a "mixed type" inhibitor of TEA accumulation in the slices.

Shown in Table IV are the effect of papaverine on the uptake of TEA and PAH in basolateral membrane vesicles isolated from kidney cortex. Papaverine caused significant inhibition

FIG. 3. Double Reciprocal Plots of TEA Accumulation in Kidney Cortical Slices against TEA Concentration

Kidney cortical slices were incubated at 37°C for 10 min in the medium containing 0.1–0.8 mM TEA with (●) or without (○) papaverine (0.05 mM). Each point is the mean of four experiments. A vertical line indicates the S.E. of the mean. Where no vertical line appears, the S.E. lies within the symbol used to designate the mean.

of TEA uptake, with no effect on the transport of PAH.

DISCUSSION

Results presented here clearly demonstrated that papaverine was taken up by kidney cortical slices (Table I) and inhibited PAH accumulation (Fig. 1). The renal transport system for organic cationic drugs such as TEA has been known to be different from that for organic anionic drugs such as PAH.^{11, 12)} Therefore, papaverine, an organic cation, is not thought to directly interfere with the carriers for PAH in renal cellular membranes. Table IV did indeed show no effect of papaverine on PAH uptake by basolateral membrane vesicles in which the carriers for PAH exist. Therefore, it seems to be evident from these data that papaverine enters kidney cells and that the drug has no direct influence on the carriers in the membranes but affects metabolisms linked to the transport process for PAH in kidney cortical slices.

Our present study was focused on the interaction of papaverine with the renal transport

TABLE III. Effect of Papaverine on Kinetic Parameters for TEA

	$V_{\max}$ (mmol/kg cell water · 15 min)	K_m (mM)
Control	3.55 ± 0.12	0.152 ± 0.02
Papaverine 0.05 mM	$2.46 \pm 0.15^b)$	$0.288 \pm 0.02^a)$

The results represent the mean $\pm$ S.E. of four experiments. Significantly different from respective control; a) $p < 0.01$, b) $p < 0.002$.

TABLE IV. Effect of Papaverine on Uptake of TEA and PAH by Basolateral Membrane Vesicles

	TEA uptake (μ mol/mg protein/15 s)	PAH uptake (pmol/mg protein/15 s)
Control	2.33 ± 0.20	27.47 ± 1.68
Papaverine 0.1 mM	$1.00 \pm 0.14^a)$	$28.29 \pm 2.78^b)$

The results represents the mean $\pm$ S.E. of five experiments.

a) Significantly different from control at $p < 0.001$.

b) Not significantly different from control.

system for TEA. TEA accumulation in the slices was inhibited by papaverine (Fig. 2) and the Lineweaver-Burke analysis for the effect of papaverine on TEA accumulation indicated that papaverine produced a "mixed type" inhibition (Fig. 3). In addition, the drug interfered prominently with TEA uptake in the isolated membrane vesicles. Although the entire nature of papaverine inhibition of TEA transport is not clear from the present results, it might be expected for papaverine to be transported at least in part by the same process as that for TEA. However, in addition to the view of the complete lack of TEA effect on papaverine depression of PAH accumulation even at about 330-fold concentration excess (Fig. 1), no abolishment of papaverine accumulation by TEA seems to indicate that the transport system for each drug is different (Table I). The results presented in this paper still do not exclude the possibility that papaverine may have severe enough affinity for the organic cationic transport system, for an extremely high concentration of TEA to produce no effect on papaverine accumulation. Therefore, the mechanisms of papaverine accumulation in kidney cortical slices is rather complex. The most reasonable conclusion to be drawn from a consideration of the data reported in this study is that papaverine may be handled, at least partly, by the same mechanisms for TEA transport and also that a different mechanism for papaverine transport may be involved in the kidney. Further work should be directed towards examining the subsystems for organic cation transport in the kidney.

In summary, it is concluded, first, that papaverine, a classical phosphodiesterase inhibitor, was taken up and produced a decrease in PAH accumulation by rat kidney cortical slices, and second, that more than one transport system for papaverine probably exists within the kidney, *i.e.*, most, if not all, of papaverine are possibly

transported by a separate mechanism from that for TEA transport.

Acknowledgement The authors are grateful to Miss. Anne M. Delany for preparing the manuscript.

REFERENCES

- 1) G. Pösch and W. R. Kukovetz: Papaverine-induced inhibition of phosphodiesterase activity in various mammalian tissues, *Life Sci.*, **10**, 133–144 (1971).
- 2) J. N. Wells, Y. J. Wu, C. E. Baird and J. G. Hardman: Phosphodiesterases from porcine coronary arteries: Inhibition of separated forms by xanthines, papaverine, and cyclic nucleotides, *Mol. Pharmacol.*, **11**, 775–783 (1975).
- 3) M. Nakashima, N. Mikuriya and M. Gemba: Effect of papaverine on organic anion transport in rat kidney cortical slices, *J. Pharm. Dyn.*, **7**, 342–345 (1984).
- 4) J. M. Irish III and J. J. Grantham, "The Kidney," 2nd ed., ed. by B. M. Brenner and F. C. Rector, Jr., W. B. Saunders Company, Philadelphia, 1981, pp.619–649.
- 5) W. O. Berndt: Use of the tissue slice technique for evaluation of renal transport processes, *Environ. Health Perspect.*, **15**, 73–88 (1976).
- 6) M. Gemba, M. Taniguchi and Y. Matsushima: Effect of bumetanide on *p*-aminohippurate transport in renal cortical slices, *J. Pharm. Dyn.*, **4**, 162–166 (1981).
- 7) J. Axelrod, R. Shofer, J. K. Inscoe, W. M. King and A. Sjoerdsma: The fate of papaverine in man and other mammals, *J. Pharmacol. Exp. Ther.*, **124**, 9–15 (1958).
- 8) K. Inui, T. Okano, M. Takano, S. Kitazawa and R. Hori: A simple method for the isolation of basolateral plasma membrane vesicles from rat kidney cortex, *Biochim. Biophys. Acta*, **647**, 150–154 (1981).
- 9) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall: Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, **193**, 265–275 (1951).
- 10) Y. Matsushima and M. Gemba: Stimulatory effect of calcium ions on *p*-aminohippurate accumulation by rat kidney cortical slices, *Renal Physiol.*, **4**, 191–198 (1981).
- 11) A. Farah and B. Rennick: Studies on the renal tubular transport of tetraethylammonium ion in renal slices of the dog, *J. Pharmacol. Exp. Ther.*, **117**, 478–487 (1956).
- 12) B. R. Rennick: Renal tubule transport of organic cations, *Am. J. Physiol.*, **240**, F83–F89 (1981).