INFLUENCE OF PAPAVERINE ON CYCLIC NUCLEOTIDE LEVEL AND CELLULAR METABOLISM IN RAT KIDNEY CORTEX IN TERMS OF ITS INHIBITORY EFFECT ON $p$-AMINOHIPPURATE TRANSPORT

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We attempted to determine whether there is a possible link between the effect of papaverine on $p$-aminohippurate (PAH) accumulation, on cyclic nucleotide content and on certain other cellular functional parameters in rat kidney cortical slices in vitro. Papaverine at a concentration of 0.1 mM almost completely inhibited PAH accumulation in the slices. However, cyclic guanosine 3', 5'-monophosphate (cyclic GMP) and cyclic adenosine 3', 5'-monophosphate (cyclic AMP) levels in the slices were not significantly affected by papaverine at 0.1 mM, though papaverine at a concentration of 1 mM increased the cyclic GMP level without affecting the cyclic AMP level. Papaverine (0.1 mM) produced a decrease in the sodium gradient and in the adenosine triphosphate (ATP) level in the slices. Calcium uptake by mitochondria, isolated from kidney cortex, was apparently decreased in the presence of 0.1 mM papaverine. These results suggest that the inhibition of phosphodiesterase probably does not explain the action of papaverine on PAH accumulation in the slices. The inhibition of PAH accumulation by papaverine is partly a reflection of the fall in the sodium gradient in the slices treated with papaverine. In addition, a depression of ATP level in the slices and an inhibition of mitochondrial calcium uptake may be related to a possible mechanism of action of papaverine on PAH accumulation.

Keywords — papaverine; $p$-aminohippurate; ATP content; mitochondrial calcium uptake; cyclic nucleotide; organic anion transport; kidney cortex

INTRODUCTION

The requirement for cyclic nucleotides, such as cyclic adenosine 3', 5'-monophosphate (cyclic AMP) and cyclic guanosine 3', 5'-monophosphate (cyclic GMP) on the regulation of cellular metabolism is well established, but the effects of these nucleotides on the renal transport system for organic anions are still not clear. In addition, the effect of cyclic AMP on the transport system remains controversial. It has been reported that cyclic AMP decreases accumulation of $p$-aminohippurate (PAH), a representative of organic anions, in rabbit kidney cortical slices. On the other hand, cyclic AMP at 1 mM inhibited PAH accumulation in the slices whereas cyclic AMP at 0.1 mM increased the accumulation. Cyclic GMP was shown in our previous study to depress PAH accumulation in rat kidney cortical slices.

Recently, we reported that papaverine inhibited PAH accumulation in rat kidney cortical slices and that the drug was taken up against a concentration gradient by the slices. Papaverine is well-known as a phosphodiesterase inhibitor, but we are unaware of any reports describing the effect of papaverine on renal cyclic nucleotide content.

The present study was undertaken to elucidate how papaverine affects the contents of cyclic GMP and cyclic AMP in relation to the inhibition of PAH accumulation by the drug in rat kidney cortical slices. Theophylline and 1-methyl-3-isobutylxanthine (MIBX), inhibitors of phosphodiesterases, have been demonstrated to suppress PAH accumulation in renal slices. Therefore, we conducted the present experiments comparing papaverine with theophylline and MIBX effects on PAH accumulation and the cyclic nucleotide contents in the slices. An additional aim of the present study was to examine the effect of papaverine on other cellular parameters related to PAH transport using kidney cortical slices and mitochondria.

MATERIALS AND METHODS

Preparation of Kidney Cortical Slices — Male Sprague-Dawley rats, weighing 200–300 g, were sacrificed by decapitation, then, bled. The kidneys were removed, placed in ice-cold isotonic saline and decapsulated. The kidney cortex
was placed on an ice-cold petri dish and cut into slices (about 0.3—0.5 mm in thickness) with a razor blade. The slices 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 Heps (N'-2-hydroxyethylpiperazine-N"-2-ethanesulfonic acid) buffer, pH 7.4, for about 20 min before use.

**Incubation for PAH Accumulation** — About 150 mg of the slices were incubated at 37 °C in 10 ml of incubation medium. The composition of the medium was identical to that described above, except that it additionally contained 0.074 mM PAH and 1% inulin which was added to estimate the inulin (extracellular) space of the slices. The medium was continually gassed with 100% oxygen. In our previous report, the time needed for steady state accumulation of PAH in the slices was found to be 30 min and this incubation time was selected for the present study. After incubation, some of the slices were blotted on a piece of filter paper, weighed and homogenized with 10% trichloroacetic acid (TCA). The incubation medium was also treated with 10% TCA. Supernatant fluids, obtained after centrifugation of the homogenate and medium, were assayed spectrophotometrically for PAH and inulin by the methods of Bratton and Marshall and Roe et al., respectively.

The intracellular concentration (S) of PAH in the slices was determined on the basis of the measurement of intracellular water. The intracellular PAH and water were calculated by correcting contents for those found in the inulin space of the slices from the total content in the slices. The total water content of the slices was the difference in weight before and after desiccation at 100 °C overnight. The accumulation of PAH (S/M) was calculated by the division of S by the medium concentration (M) of PAH.

**Determination of Cyclic Nucleotide, Sodium and Adenosine Triphosphate (ATP) Contents in the Slices** — The slices were incubated at 37 °C for 30 min in the same medium described above. Incubation was terminated by placing the slices in liquid nitrogen for measurement of the nucleotides. After the frozen slices were weighed, they were homogenized with Microtron (Nitro NS-300) in cold 6% perchloric acid (PCA) and the homogenate was centrifuged. PCA in the supernatant fluid was neutralized with KOH. Cyclic GMP and cyclic AMP in tissue samples were measured by a radio-immunoassay with cyclic GMP and cyclic AMP kits obtained from Yamasa Shoyu (Chyoshi, Japan). The methods employed were essentially similar to those described by Steiner et al. The radioactivity of 125I was measured using an autowellgamma system (Aloka ARC-301). Sodium and ATP in the incubated slices were determined as described previously with a flame photometer (Hitachi 205D) and an ATP photometer (model 2000, JRB Co.), respectively.

**Mitochondrial Calcium Uptake and Oxygen Consumption** — Kidney cortical mitochondria were prepared according to a standard procedure as described previously. The mitochondria were incubated at 37 °C in medium containing 140 mM KCl, 10 mM tris (hydroxymethyl) aminomethane (Tris)-HCl buffer (pH 7.4), 50 mM sucrose, 2 mM MgCl₂, 0.5 mM CaCl₂ and 2 mM ATP (Tris salt). The medium additionally contained 15.6 mM succinate (Tris salt) as a respiratory substrate for the measurement of oxygen consumption which was measured with a conventional Warburg apparatus. Calcium uptake by mitochondria was assayed with a rapid filtration-method through membrane filters (Sartorius 11305, pore size 0.6 μm) as described previously. Calcium extracted from mitochondria with 61% nitric acid was determined with an atomic absorption spectrophotometer (Hitachi 170-10). Protein of mitochondria was assayed by the method of Lowry et al. using bovine serum albumin as a standard.

**Statistics** — Statistical significance was evaluated by an analysis of variance combined with Dunnnett’s multiple range tests. A difference of p values less than 0.05 was considered significant.

**RESULTS**

**PAH Accumulation**

Figure 1 shows that papaverine at a concentration of 0.01 mM significantly inhibited PAH accumulation in rat kidney cortical slices. The efficacy of theophylline on PAH accumulation in the slices was undetectable at 0.01 mM, but detectable at 0.1 mM. MIBX inhibited PAH accumulation in the slices at the two concentrations tested. This inhibitory action of theophylline and MIBX on PAH accumulation was small when compared with that of papaverine. The changes in water content and extracellular space
of the slices during incubation remained unaltered in the presence of the compounds tested, when compared with those in control slices (data not shown). Therefore, the inhibitory actions by those compounds on PAH accumulation were not due to a secondary effect, via the effect on water distribution in the slices.

**Cyclic Nucleotide Content**

Experiments were carried out to compare the effect of papaverine, theophylline and MIBX on the contents of cyclic GMP and cyclic AMP in the kidney cortical slices. A summary of the levels of cyclic GMP and cyclic AMP with or without these compounds is provided in Figs. 2 and 3. Papaverine (0.1 mM) had no significant effect on the levels of both cyclic nucleotides in the slices. Theophylline (0.1 mM) also had no significant effect on either the cyclic GMP or cyclic AMP level in the slices. Papaverine (1 mM) did not increase the cyclic AMP level but increased the cyclic GMP level in the slices. In contrast, theophylline (1 mM) increased the cyclic AMP level without affecting the cyclic GMP level in the slices. MIBX at 0.1 mM produced a significant increase in the levels of cyclic GMP and cyclic AMP in the slices.

**Sodium and ATP Contents**

We examined whether the inhibitory effect of papaverine on PAH accumulation in the slices is due to the effect on sodium concentration and ATP content in the slices. As shown in Table I, the intracellular sodium concentration in the slices was increased by papaverine (0.1 mM) in a medium, so the sodium gradient was decreased. The increase in sodium concentration was approximately the same whether by the addition of 0.1 mM papaverine or by 0.2 mM ouabain. However, papaverine (0.1 mM) almost completely inhibited PAH accumulation while

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**Fig. 1. Effect of Papaverine, Theophylline and MIBX on PAH Accumulation in Rat Kidney Cortical Slices**

The slices were incubated for 30 min with or without each agent at the indicated concentration in mM. Each column represents the mean ± S.E. of at least four experiments. a) p<0.05, b) p<0.01, significantly different from the control.

**Fig. 2. Effect of Papaverine, Theophylline and MIBX on Cyclic GMP Content in Rat Kidney Cortical Slices**

The slices were incubated for 20 min without the addition of any agent, and then incubated for a consecutive 10 min with each agent at the indicated concentration in mM. Each column represents the mean ± S.E. of at least four experiments. a) p<0.01, significantly different from the control.

**Fig. 3. Effect of Papaverine, Theophylline and MIBX on Cyclic AMP Content in Rat Kidney Cortical Slices**

The slices were incubated for 20 min without the addition of any agent and then incubated for a consecutive 10 min with each agent at the indicated concentration in mM. Each column represents the mean ± S.E. of at least four experiments except for three in the case of 0.1 mM MIBX effect. a) p<0.05, significantly different from the control.
### TABLE I. Effect of Papaverine on Sodium Concentration, ATP Content and PAH Accumulation in Rat Kidney Cortical Slices

<table>
<thead>
<tr>
<th>Additions</th>
<th>Sodium concentration (mmol/kg cell water)</th>
<th>PAH accumulation (S/M)</th>
<th>ATP content (mmol/kg cell water)</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>60.5 ± 5.0</td>
<td>13.94 ± 0.71</td>
<td>1.81 ± 0.15</td>
</tr>
<tr>
<td>Papaverine 0.1 mM</td>
<td>80.9 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.95 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.13 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ouabain</td>
<td>81.9 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.22 ± 0.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
</tr>
</tbody>
</table>

The slices were incubated at 37°C for 30 min in the presence of papaverine (0.1 mM) or ouabain (0.2 mM) which affected intracellular sodium concentration almost to the same extent. Each value represents the mean ± S.E. of at least four experiments. 

<sup>a</sup> p < 0.05,  
<sup>b</sup> p < 0.02 and  
<sup>c</sup> p < 0.005, when compared with the respective None control.

### TABLE II. Effect of Papaverine on Calcium Uptake and Oxygen Consumption by Rat Kidney Cortical Mitochondria

<table>
<thead>
<tr>
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<th>Calcium uptake (nmol/mg protein/min)</th>
<th>Oxygen consumption (μl oxygen/mg protein/min)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>243.3 ± 26.6 (6)</td>
<td>1.53 ± 0.13 (4)</td>
</tr>
<tr>
<td>Papaverine 0.01 mM</td>
<td>186.3 ± 12.2 N.S. (4)</td>
<td>—</td>
</tr>
<tr>
<td>Papaverine 0.1 mM</td>
<td>140.0 ± 19.9&lt;sup&gt;d&lt;/sup&gt; (6)</td>
<td>1.61 ± 0.17 N.S. (4)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. The numbers in parentheses are the number of experiments. 

<sup>d</sup> p < 0.01, when compared with control. N.S.: Not significantly different from control.

ouabain (0.2 mM) only slightly inhibited the accumulation. The ATP level in the slices was decreased by approximately 40% in the presence of 0.1 mM papaverine.

Table II shows that 0.1 mM papaverine inhibited calcium uptake without affecting oxygen consumption by mitochondria.

### DISCUSSION

The present experiments verified the inhibition of PAH accumulation by papaverine as previously reported.<sup>5</sup> Since PAH accumulation in renal tissues is affected by cyclic nucleotides,<sup>1–4</sup> we thought that the inhibitory effect of papaverine on PAH accumulation in the slices might be expected to result from its well-known activity as an inhibitor of phosphodiesterases, resulting in an increase in cyclic nucleotide contents in the tissues. The effect of papaverine on cyclic nucleotides in kidney cortical tissues has not been reported, though it has been suggested that papaverine inhibits phosphodiesterase of renal tissues.<sup>22</sup> It has been reported that a tissue preincubation is neccessary for a maximum increase in cyclic nucleotide levels.<sup>23</sup> Therefore, in the experiments designed to confirm the effects of the three compounds on cyclic nucleotide levels, the slices were incubated for 30 min in total, which consisted of a 20 min preincubation period without any test compounds and a consecutive 10 min incubation period with one of the three compounds. Papaverine and theophylline, at 0.1 mM each, affected neither cyclic GMP or cyclic AMP level in the slices, but a higher concentration of papaverine (1 mM) significantly increased the level of cyclic GMP but not cyclic AMP and theophylline (1 mM) significantly increased the level of cyclic AMP but not cyclic GMP. Both cyclic nucleotide levels were elevated by MIBX (0.1 mM). These drugs differentially affected the levels of the cyclic nucleotides in the slices. This suggests that papaverine, theophylline and MIBX inhibit cyclic GMP phosphodiesterase and cyclic AMP phosphodiesterase activities to different degrees in kidney cortex.

At a concentration of 0.1 mM, papaverine almost abolished PAH accumulation but theophylline and MIBX were found to be less effective. The rank order of inhibition of PAH accumulation in the slices as judged by the concentration used (0.1 mM) (papaverine >> MIBX > theophylline) did not correlate with the rank order of increase in cyclic GMP (MIBX > papa-
verine >theophylline) and in cyclic AMP (MIBX >theophylline ≈ papaverine). In addition to their actions as inhibitors of phosphodiesterases, theophylline and MIBX competitively decrease PAH accumulation in the slices as suggested in earlier studies.\textsuperscript{1,11} It is possible that the effect of theophylline at 0.1 mM and MIBX at 0.01 mM on PAH accumulation in the slices was due to a competitive inhibition, while the competition with PAH transport and the inhibition of phosphodiesterases, collectively, may be responsible for the inhibition of PAH accumulation by 0.1 mM MIBX. Recently, we demonstrated that papaverine was transported against a concentration gradient from the bathing media to the slices.\textsuperscript{39} However, the inhibition of PAH accumulation by papaverine probably is of a non-competitive type, since papaverine is an organic cation, the transport system of which has been known to be different from that of organic anions such as PAH.\textsuperscript{24,25} Both cyclic nucleotide levels were not affected significantly by papaverine at a concentration of 0.1 mM which markedly depressed PAH accumulation in the slices. The inhibition of PAH accumulation by the higher concentration of papaverine (1 mM) may be, in part, due to the rise in cyclic GMP level in the presence of the drug, but the inhibition of PAH accumulation by the low concentration of papaverine (≤0.1 mM) may not be explicable in terms of inhibition of phosphodiesterases. Further study is necessary to confirm this conclusion.

Although it is not possible to explain the exact mechanism of the action of papaverine on PAH transport at present, it is clear from our results that papaverine affected sodium gradient and ATP level in the slices, which are probably implicated in the transport process of PAH in renal tissues.\textsuperscript{26–30} There was a marked difference between the slices with papaverine and with ouabain when compared with respect to PAH accumulation under the condition that sodium gradient was diminished by approximately the same amount in the presence of the both compounds. These results suggest that the inhibitory action of papaverine on PAH accumulation cannot be explained only by the decrease in sodium gradient by the drug.

The decrease in ATP content in the slices when treated with papaverine suggests that mitochondrial disfunction may be involved in papaverine effect. To ascertain this we studied the papaverine action on the functions of isolated kidney cortical mitochondria. It has been reported that oxygen consumption by rat liver mitochondria was unaffected by papaverine with succinate as substrate.\textsuperscript{31} In the present study, papaverine had no effect on oxygen consumption by kidney cortical mitochondria oxidizing succinate as substrate. However, papaverine at 0.1 mM decreased calcium uptake to mitochondrial storage, though the inhibition of calcium uptake by the drug at 0.01 mM was not significant. The disturbance of calcium uptake by mitochondria may raise the cytoplasmic calcium concentration.\textsuperscript{32} There are several studies with renal slices and tubules indicating that calcium is important for organic anion transport though its mechanism of action is unknown. The removal of calcium from the incubation medium inhibited the transport of organic anion such as phenol red and chlorophenol red across the luminal membrane of tubules.\textsuperscript{33,34} The omission of calcium from the medium also reduced PAH accumulation in kidney cortical slices.\textsuperscript{12,35} Normal calcium entry across renal tubular membranes, in addition to the cytosolic calcium level, appeared to be important for PAH secretion by tubules.\textsuperscript{36}

The present findings suggest that the decreased sodium gradient and the depressed ATP level are probably attributable in part to the decreased PAH accumulation in the slices in the presence of low concentration of papaverine (≤0.1 mM). The rise of cyclic GMP level, in addition to above factors, may be involved in the inhibition of PAH accumulation by higher concentration of papaverine (>0.1 mM). It is also significant to note the possibility that mitochondrial calcium uptake may play a role in the mechanism of papaverine action on PAH transport in renal tissues in view of the hypothesis that cellular calcium may be associated with the activity of PAH transport. However, no direct causal relationship was established in the present study. Further experiments are required to assess this possibility.

REFERENCES

2) J. Maxild: Effect of externally added ATP and related


16) A. L. Steiner, A. S. Pagliara, L. R. Chase and D. M. Kipnis: Radioimmunoassay for cyclic nucleotides. II. Adenosine 3', 5'-monophosphate and guanosine 3', 5'-monophosphate in mammalian tissues and body fluids, \textit{J. Biol. Chem.}, 247, 1114–1120 (1972).


34) S. K. Hong and R. P. Forster: Further observations on
