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Nephron Distribution of Total Low Km Cyclic AMP Phosphodiesterase in Mouse, Rat and Rabbit Kidney

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KUSANO, E., YOSHIDA, I., TAKEDA, S., HOMMA, S., YUSUFI, A.N.K., DOUSA, T.P. and ASANO, Y. *Nephron Distribution of Total Low Km Cyclic AMP Phosphodiesterase in Mouse, Rat and Rabbit Kidney.* Tohoku J. Exp. Med., 2001, **193**(3), 207-220 —— The activity of cAMP degradation enzyme, cAMP phosphodiesterase (cAMP PDE), in renal tubules is a critically important factor in determining cellular cAMP levels, particularly in response to hormones. In this study we examine the nephron distribution of cAMP PDE activity in the mouse, rat and rabbit kidney and important cellular regulators of cAMP PDE, namely calmodulin and adenosine triphosphate (ATP). We assayed total low Km cAMP PDE in microdissected tubule segments, using 10^{-6} M (^{3}H)-cAMP as a substrate. Activities were expressed in fentomol cAMP hydrolyzed per minute per mm tubular length or per one glomerulus. The content of ATP was measured in outer medullary collecting duct and medullary thick ascending limb of Henle's loop with microbioluminescence assay using firefly luciferase. In mouse kidney, cAMP PDE was significantly higher in all tubular segments compared to glomerulus. Proximal convoluted tubule, proximal straight tubule, medullary thick ascending limb of Henle's loop (mTAL), and outer medullary collecting duct (OMCD) had intermediated activity. Greater cAMP PDE activity was detected in cortical ascending limb of Henle's loop (cTAL), cortical collecting duct and in distal convoluted tubule (DCT). The highest activity was found in connecting tubules. In rat, nephron distribution of cAMP PDE activities was similar to mouse, except that activity in glomeruli was higher than in mouse glomeruli. In rabbit, nephron distribution of cAMP PDE activities was different from those of mouse and rat. There was no single prominent segment with high cAMP PDE activity. DCT and cTAL showed low enzyme activity. Overall, the highest cAMP PDE

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activities were measured in the mouse and the lowest were measured in the rabbit nephrons, with those of rat nephron showing an intermediate activity. The maximum effective dose of the calmodulin antagonist, trifluoperazine (200 μM), inhibited cAMP PDE in all nephron segments from the rat kidney. However, there is no statistical significance of its inhibition among nephron segments. In OMCD and mTAL of the rat kidney, cAMP PDE activity was inhibited by ATP (5 mM$\sim$10 mM) which is far beyond the physiological concentartion of ATP in normal epithelial cell. Actual determinations of ATP in mTAL and OMCD were 0.1 mM and 0.17 mM, respectively. These observations show that distal segments of tubules have more active catabolism of cAMP than proximal segments. cAMP PDE in each nephron segment appear to be almost equally dependent on trifluoperazine-sensitive pathway that may reflect the Ca^{2+}-calmodulin system. Cellular concentration of ATP might not be involved in the regulation of the total low Km cAMP PDE activity in rat mTAL and OMCD. ——— nephron segement; cAMP phosphdiesterase; calmodulin; trifluoperazine; ATP

The importance of the structural and functional heterogeneity of the renal tubular system is well recognized (Burg et al. 1966). With the use of a micromethod assay of adenylate cyclase activity introduced by by Imbert et al. (1975), this enzyme was thoroughly examined in virtually all nephron segments, in terms of basal activity and responsiveness to hormonal agents and drugs. Each hormone and agent were found to stimulate adenylate cyclase activity in well-localized segments of tubule according to a highly specific and reproducible pattern. There have been several reviews concerned with biochemical heterogeniety of nephrons in terms of cAMP generation.

On the other hand, cyclic-3′, 5′-AMP (cAMP) catabolism mediated by cAMP phosphodiesterase (cAMP PDE)1 has not been well studied in specific nephron segments in a similar way. The rate of cAMP formation and the intracellular cAMP concentration are dependent on not only the rate of biosynthesis mediated by adenylate cyclase but also on the rate of hydrolysis by a specific enzyme, cAMP PDE, and on the rate of efflux of cAMP from the cell. The important role of cAMP PDE in tubular response to vasopressin is exemplified in mice with hereditary nephrogenic diabetes insipidus in which excessively high cAMP PDE in OMCD prevents adequate accumulation of cAMP in response to vasopressin (Jackson et al. 1980a; Kusano et al. 1986). Likewise, evidence to date suggests that blunted response of collecting ducts to vasopressin in adrenal insufficiency may be due to increased activity of cAMP catabolizing enzyme and treatment with glucocorticoid could correct the defect by diminishing cAMP PDE activity (Jackson et al. 1983).

In spite of potential importance of cAMP PDE, this enzyme was relatively little studied unlike adenylate cyclase in each nephron segment. Therefore, in the present study, we measured total low Km cAMP PDE activity in most major nephron segments from mouse, rat and rabbit kidney up to OMCD. In addition, we examined the effect of some regulatory factors such as Ca^{2+}CaM and ATP (Dousa and Rychlik 1970) on cAMP PDE in mTAL, OMCD, and other nephron segments.

Materials and Methods

Tissue preparation and microdissection of tubules

Kidneys were obtained from male C-57 mice (25–50 g), Sprague-Dawley rats (200–250 g) and New Zealand white rabbit (2–3 kg). All animals were fed with the standard laboratory diet and had free access to tap water prior to sacrifice. Except for several modifications dictated by the difference in kidney size, the procedure for kidney perfusion and microdissection described below was similar for the three species (Jackson et al. 1980a; Kusano et al. 1984; Yoshida et al. 1997).

The animals were lightly anesthetized with pentobarbital (rats 6 mg/100 g body wt, i.p., mice 10 mg/100 g body wt, i.p. and rabbit 35 mg/kg body wt, i.v., respectively) and the left kidney of mouse and rat was perfused in situ via the abdominal aorta, while the left kidney of rabbit was perfused in situ via the left renal artery with a cold aerated collagenase medium (for composition, see below) containing sodium heparin (20 U/ml). Following perfusion, the kidney was quickly removed and sliced with a razor blade along the cortico-medullary axis. The slices were then incubated in aerated collagenase medium at 30–35°C for 30–40 minutes, depending on the activity of collagenase, the specific activity of which varied from (125 to 250 U/mg). After incubation, the slices were thoroughly rinsed in "microdissection medium" (for composition, see below) and transferred to Petri dishes for microdissection. All subsequent steps, including microdissection, were performed at 0–4°C.

The various segments of the nephron were identified using established criteria originally described by Morel et al. (1981) and in our previous studies (Yoshida et al. 1997) under a stereomicroscope (magnification $\times$ 20–40) and their identity was confirmed by examinations of photograph pictures taken at higher magnification. Glomeruli were gathered from a suspension of digested kidney tissue. The early part of the proximal convoluted tubule (PCT) was easily detected by typical appearance and in relation to its attached glomeruli; the proximal straight tubule (PST) was identified by its continuity with the thin descending limb of Henle's loop. The medullary (mTAL) and cortical (cTAL) segments of the thick ascending limb are bright and relatively straight, and were easily distinguishable from the adjacent tubules by their appearance and diameter. The distal convoluted tubule, which generally defined as the nephron portion between the macula densa and cortical collecting tubule. This segment was subdivided to distal convoluted tubule proper (DCT) and connecting tubule (CNT) as defined by Kaissling and Kriz (1979) and functionally characterized by Imai (1979).

The cortical (CCD) and outer medullary (OMCD) portions of the collecting tubule were easily identifiable by their diameter, weak light reflex in the dissecting dish, and in the case of

Footnote

1 PDE denotes cyclic-3′, 5′-nucleotides phosphodiesterase in general. cAMP PDE and cGMP PDE denote PDE activity using cAMP and cGMP as substrate, respectively. In this report, the PDE isozymes are classified as proposed by international committee in 1994 (Beavo et al. 1994; Beavo 1995). According to this nomenclature, the superfamily of mammalian PDE isozymes consists of nine gene families denoted PDE1 through PDE9. PDE1 is the family of PDE isozymes dependent on Ca^{2+} and calmodulin. In this classfication system, gene families of the PDE are designated by Arabic numerals, for example, PDE4 instead of formerly PDE-IV. This nomenclature is also in conformity with the Gene Bank nomenclature system and identifies PDE isozymes by vertebrate species, gene family, single gene product, and isoform-splice variant.

G; glomerulus, PCT; proximal convoluted tubule, PST; proximal straight tubule, mTAL; medullary thick ascending limb of Henle's loop, cTAL; cortical thick ascending limb of Henle's loop, DCT; distal convoluted tubule, CNT; connecting tubule, CCD; cortical collecting duct, OMCD, outer medullary collecting duct.

CCD, by its branching to the other nephrons.

Dissected segments were aspirated in a small droplet of "microdissection medium" and transferred onto small round pieces (about 3 mm^2) of glass cover slip. All tubule segments were then photographed (magnification 40×) and tubular lengths were measured from the photographs by using a planner measure.

Mixed cortical tubules preparation

Mixed cortical tubules were prepared from the kidneys of Sprague-Dawley rats according to the procedure described by Vinay et al. (1981). Kidneys from four to six rats were used for one preparation. Briefly, removed kidneys were placed in 30 ml of ice-cold Krebs-Henseleit saline (KHS, pH 7.40, composition see solutions and materials) previously gassed at 20°C with 95% O_2/5% CO_2 for 30 minutes. The kidneys were cut in half and the medulla was carefully dissected out. The resultant chips of cortex were placed in 20 ml of ice-cold KHS and were sliced with a Stadie-Riggs microtome. The slices were pooled and washed 3 times with 30 ml of the solution and resuspended in 10 ml of KHS containing 0.15 g/100 ml of collagenase (Type I, Sigma) and 0.5 ml of 10% (wt/vol) bovine albumin (fraction V, Miles Laboratories, El Kart, IN, USA) dialyzed for 48 hours. The slices in collagenase were then transferred into a 250-ml Erlenmeyer flask previously coated with silicone (dimethyl dichlorosilane) to prevent adhesion of the tissue to the walls. The Erlenmeyer flask was then gassed for 2 minutes with 95% O_2/5% CO_2, closed with a rubber stopper, and placed in a 40 rpm shaking bath with temperature control at 37°C for 45 minutes. At the end of the digestion procedure, 30 ml of ice-cold KHS was added and the suspension was gently shaken to disperse the fragments of tissue. The whole suspension was filtered through a tea strainer to remove the collagen fibers. The tissue suspension was then gently centrifuged (60 g) in a 50 ml tube for 30 seconds. The supernatant was discarded and the tissue rapidly resuspended in 30 ml of ice-cold KHS. This washing procedure was repeated 3 times.

After the last washing, the pellet was resuspended in 5% albumin for 5 minutes (4°C), spun again (60 g), and the supernatant was discarded. The tissue was now resuspended in 50% Percoll solution (Pharmacia Fine Chemicals, Montreal, Composition see below). The whole Percoll solution was gassed and was placed on crushed ice and chilled to 4°C before use. The tissue pellet was resuspended in 120 ml of ice-cold Percoll, which was divided into four 30-ml fractions placed in spun for 30 minutes at 4°C and 15 000 rpm on a B-20 Beckman Centrifuge equipped with a fixed-angle rotor head, ensuring about 12 200 g. This procedure established a gradient of density varing between 1.05 and 1.35. The density gradient was monitored with a refractometer. The tissue was then separated into four distinct bands, F1-F4. The bands were carefully removed from the Percoll gradient with an automatic 1 ml Eppendorf pipette. Each fraction was then resuspended in ice-cold KHS and washed 3 times as previously described. After the last centrifugation, the tissue was resuspended in 10 vol of ice-cold 0.25 M sucrose containing 5 mM Tris, pH 7.4 and 1 mM dithiothreitol. The tissue suspended in sucrose was then homogenized gently in a teflon pestle-glass homogenizer (size 0; clearance 0.05 to 0.1 mm; A.H. Thomas, Co., Philadelphia, PA, USA) using five strokes. Aliquots of mixed cortical tubule homogenate or microdissected tubules were quickly frozen in dry ice and stored at −80°C until assayed.

Assays

To minimize day-to-day variations between assays and preparative procedures, various nephron segments were dissected from one kidney, incubated and assayed simultaneously. Likewise, when the actions of drugs were studied, tubules from the same animal were incubated with or without test agents within the same

experiment.

The tubular cells were "permeabilized" prior to the assay for cAMP PDE by a combination of freezing and hypoosmotic shock. The microdissection medium was aspirated from each sample and replaced with 0.25 μl "hypoosmotic medium" (composition, given below). All samples were then frozen rapidly by placing them on a block of dry-ice and stored at -80°C overnight. Before assay, samples were allowed to thaw at 0-4°C and then used for determination of enzyme activities. As described in detail previously (Tetsuka et al. 1995; Yoshida et al. 1997), tubular samples were incubated for 10 min at 37°C in a final volume of 2.5 μl consisting of 10 mM $MgSO_4$, 0.1 mM EDTA, 50 mM Tris HCL, pH 8.0 ("Mg-EDTA-Tris buffer"), with a final substrate concentration of 10^{-6}M [^{3}H]cAMP (4×10^4 cpm/sample)

The reaction was terminated by placing the bacterial slides with samples on a block of dry ice. The samples, still frozen on glass cover slips fragments, were quickly transferred with forceps into the bottom of glass test tube (12$\times$ 75 mm), followed by two successive 50 μl of 5′-nucleotidase (rattlesnake venom from Crotalus atrox, Sigma Co., 1 mg/ml) were added to each tube and incubated for a further 15 minutes at 37°C. Nucleotides were separated from nucleosides on columns of QAE-Sephadex (Pharmacia Fine Chemicals, Piscataway, NJ, USA).

In preliminary experiments of PCT and DCT of mouse kidney, it was determined that, under the conditions described above, cAMP hydrolysis is linearly proportional to tubular length (up to at least a total of 3.5 mm/sample) and also linearly proportional to time (up to at least 20 minutes). Likewise, in OMCD and mTAL of rat kidney and mouse kidney linearity of tubular length dependency and time dependency of cAMP hydrolysis were determined in our laboratory (Jackson et al. 1980). Consequently, specific activity of cAMP PDE was expressed in femtomoles of cAMP hydrolyzed per minutes per millimeter of tubule length. In the case of glomeruli, the cAMP PDE activity was expressed fmoles cAMP hydrolyzed per one glomerulus. Protein contents in glomeruli and proximal convoluted tubules from kidneys from mouse, rat and rabbit were determined by microadaptation of Lowry's method (Table 1). In mouse kidney, protein content was 0.03 μg per glomerulus and 0.286 μg per 1 mm of PCT. Therefore, assuming similar random composition of cellular proteins, cellular mass of 1 glomurulus corresponded to 0.12 mm of PCT. Likewise, 1 glomerulus of rat and rabbit kidney corresponded to 0.26 mm and 0.31 mm of PCT, respectively. cAMP PDE in homogenate of mixed proximal tubules, was incubated with 10^{-6}M of [^{3}H] cAMP in an incubation mixture (total volume 50 μl) containing: 10 mM $MgSO_4$, 50 mM Tris-Base (pH 8.0), 0.3 mM EGTA, unless specified otherwise. After 10 minutes incubation period at 37°C, the reaction was stopped by heating at 95°C for 3 minutes, and the [^{3}H]5′-nucleotide products were converted to [^{3}H] nucleosides by incubation with an excess of 5′-nucleotidase.

TABLE 1. *Protein content of PCT and G from mouse, rat and rabbit kidney*

	Mouse	Rat	Rabbit
PCT (μg/mm)	0.286$\pm$0.014*	0.368$\pm$0.027	0.291$\pm$0.022
n	25	11	16
G (μg/G)	0.035$\pm$0.006	0.097$\pm$0.006	0.090$\pm$0.004
n	9	19	10

*Mean$\pm$S.E.M. *n*, number of samples.

Enzymatic reaction was linear with time for at least up to 25 minutes and linear up to 30 μg of protein per tube. The enzyme activity was expressed in picomoles of cAMP hydrolyzed per minute per milligram of protein. Protein was measured by the Lowry method.

Determination of ATP

ATP was measured with a micro-bioluminescence assay adapted in our laboratory for sampling of dissected tubules. This method involves the use of a buffered, highly purified firefly luciferase-luciferin reagent (Packard Instrument Co., Inc., Downers Grove, Il; Picozyme ATP kit, No.). Briefly, ATP is converted to 5′-AMP in the presence of excess luciferase enzyme with the concomitant release of light (one photon of light per molecule of ATP [-]). The light produced from this reaction was measured using a Packard Pico-Lite (model 6100) luminometer. Standards were prepared by adding 10 μl of ATP standard solution (serially diluted with a 20 mM Tris, $MgSO_4$ buffer, pH 7.75, (Packard #6016820) to 40 μl of enzyme (i.e., firefly luciferase-luciferin reagent). Samples were prepared by adding 15 μl of Tris, $MgSO_4$ buffer plus 15 μl of ATP extraction reagent (Packard #6016740) to a test tube containing nephron segments frozen in 2.0 μl of incubation media. After mixing, 10 μl of the sample solution was added to 40 ml enzyme solution. ATP titers as little as 1.0 fmole/tube could be accurately detected by this method. Total counts were proportional to ATP concentration over the working range of 1.0-1000.0 fmol/tube.

Solutions and materials

The collagenase medium consisted of Medium 199 (Grand Island, NY, USA) plus collagenase (0.1%), hyaluronidase (0.1%) and bovine serum albumin (0.1%). Microdissection medium was Medium 199 without the above additives and also without phenol red indicator. Hypoosmotic medium contained (in final concentrations): 1 mM $MgCl_2$, 0.25 mM EDTA, 0.1% bovine serum albumin, and 1 mM Tris HCl (pH 7.4). Modified Krebs Ringer Buffer consisted of 215 mM NaCl, 350 mM Urea, 5 mM KCl, 1.2 mM $MgSO_4$, 1.0 mM CaCl2, 10 mM sodium acetate, 10 mM glucose, 20 mM Tris, 2.0 mM NaH_2PO_4, pH 7.4. Krebs Henseleit saline contained (in final concentrations): 28 mM HCO_3, 140 mEq/liter Na, 117 mEq/liter Cl, 4.9 mEq/liter K, 1.2 mM PO_4, 2 mM Ca. 50% of Percoll solution contained the same salts as KHS except for adding 50% of Percoll. Collagenase (type I), hyaluronidase (type I), bovine serum albumin, Trifluroperazine, ATP, cAMP, 5′-nucleotidase (from Crotalus atrox) were products of Sigma Chemical Co. (St. Louis, MO, USA). Sodium heparin (100 USP U/ml) was a product of Abbott Laboratories (North Chicago, IL, USA.

[^{3}H]cAMP (30-50 Ci/mmol) was from New England Nuclear (Boston, MS USA). All other compounds and reagents were of the highest quality available from standard suppliers.

Statistics

The results are expressed as mean $\pm$S.E.M. Data were analyzed by an analysis of variance combined with Fisher's Protected Least Significant Difference (Fisher's PLSD). Differences with $p < 0.05$ were considered to be significant.

Results

Nephron distribution of cAMP PDE in mouse, rat and rabbit kidney

The profile of total low Km cAMP PDE activity along the mouse nephron is shown in Fig. 1. In mouse kidney as shown in open column, cAMP PDE activity in glomerulus was significantly lower (at least fourfold) than in all tubular segments tested based on the mm tubular length vs. per one glomerulus. However, these differences were canceled when cAMP PDE activity was expressed on protein basis.

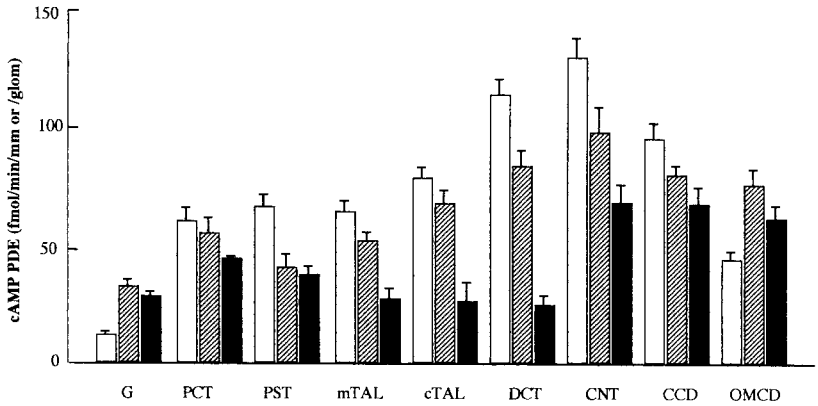

Fig. 1. Profile of cAMP PDE activity along mouse, rat and rabbit nephron.
□, cAMP PDE activity in mouse kidney; ▨, cAMP PDE activity in rat kidney; ■, cAMP PDE activity in rabbit kidney.
Ordinate: fmol cAMP hydrolyzed per mm tubular length or one glomerulus per one minute.
Abscissa: Denote each nephron segment.
Each bar represents mean ±S.E.M. of 8–27 samples from 3–4 animals.
G, glomerulus; PCT, proximal convoluted tubule; PST, proximal straight tubule; mTAL, medullary thick ascending limb of Henle's loop; cTAL, cortical thick ascending limb of Henle's loop; DCT, distal convoluted tubule; CNT, connecting tubule; CCD, cortical collecting duct; OMCD, outer medullary collecting duct.

From tubular segments, PCT, PST, mTAL, and OMCD had about intermediated activity (range 44.5–66.7 fmol cAMP/mm/min). Greater cAMP PDE activity was detected in some distal tubular segments, namely cTAL, CCD and DCT (range 79.1–115.2 fmol cAMP/min/mm). The highest activity (131±7.4 fmol cAMP/min/mm) was found in CNT.

Distribution of cAMP PDE activities along the rat nephron was basically similar to that of mouse kidney, as shown in shaded column, although the absolute activity of the enzyme was lower than that of mouse kidney except for glomerulus and medullary collecting tubule. OMCD had relatively higher activity compared to other segments (CCD, DCT) than that of mouse kidney (Fig. 1). CNT also showed highest enzyme activity as in mouse kidney.

Rabbit kidney showed quite different pattern of nephron distribution compared to mouse and rat kidney as indicated by closed column. Particularly, there was no single prominant segment with high cAMP PDE activity as CNT observed in mouse and rat kidney. Instead of that, three distal segments, namely CNT, CCD and OMCD showed equally high activities. In addition, DCT and cTAL had quite low activity although these segments showed high activities in mouse and rat kidney.

In general, absolute enzyme activity in rabbit kidney was lowest among three species of animals.

The effect of trifluoperazine

In preliminary experiments, a specific inhibition of Ca^{2+}-calmodulin sensitive cAMP PDE (PDE1) was examined in the preparation of mixed cortical tubules from rat kidney. Increasing concentration of the effect of trifluoperazine (TFP) progressively inhibited the activity of cAMP PDE and nearly maximum inhibitory effect was achieved at a concentration about 200 μM of TFP. Average inhibition rate at concentrations of 100, 200, 300 μM of TFP were 48.6, 65.6, 69.8%, respectively. Therefore, 200 μM of TFP was used to examine sensitivity of individual microdissected nephron segments. As shown in Fig. 2, 200 μM

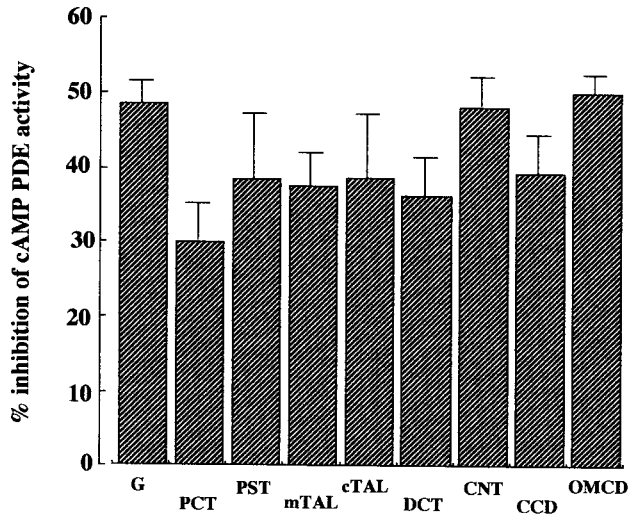

Fig. 2. Inhibition of cAMP PDE by trifluoperazine (TFP) in individual nephron segment from rat kidney.
Ordinate: Percent inhibition of cAMP PDE activity.
Abscissa: Denote each nephron segment.
Each bar represents mean ±S.E.M. of 7–18 samples from 3–4 animals.

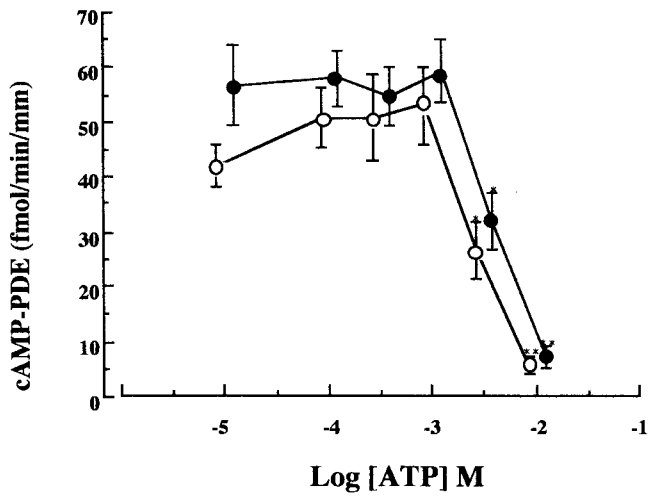

Fig. 3. Effect of ATP on cAMP PDE activity in mTAL and OMCD from rat kidney.
Ordinate: fmol cAMP hydrolyzed per mm tubular length per one minute.
Abscissa: concentration of ATP described as log [ATP]M.
Dotted line, OMCD, Solid line, mTAL.
Each point represents mean ±S.E.M. of 7–8 samples.
$^{*}p<0.05$ vs. corresponding log [ATP] $=-5$, $^{**}p<0.01$ vs. corresponding log [ATP] $=-5$.

TFP showed inhibition in all segments of the tubule tested in these experiments. Although there are some trends that higher degree of inhibition was observed in glomerulus, CNT, OMCD and the lesser degree of inhibition was found in PCT and DCT, there is no statistical significance of its inhibition among nephron segments. Therefore, the proportion of PDE1 in total cAMP PDE activity may not differ in each nephron segment.

The effect of exogenous adenosinetriphosphate

In past experiments in tissue extracted from the whole kidney, we observed that exogenous adenosinetriphospate (ATP), a substrate for cAMP generation, inhibited cAMP PDE (Dousa and Rychlik 1970). Therefore, we examined the effect of exogenous ATP on cAMP PDE activity in intact mTAL and OMCD, segments which are responsible for action of vasopressin. As shown in Fig. 3, ATP in concentration range from 5 mM to 10 mM causes prominent inhibition of cAMP PDE both in mTAL and OMCD. The extent of inhibition appears to be similar in both segments. When we measured the ATP concentrations in mTAL and OMCD, they were approximately 0.10 mM and 0.17 mM, respectively. Therefore, these cellular concentarations of ATP may not sufficient to inhibit cAMP PDE activity.

Discussion

More than 35 years ago, Sutherland and associates discovered a specific adenine nucleotide, cAMP is a cellular second messenger of hormones, autacoids, and neurotransmitters (Robinson et al. 1971). Thereafter, the question of how cAMP is synthesized by adenylate cyclase and its regulation by hormonal agents attracted immediate and enduring attention of many investigators. On the other hand, c AMP catabolizing enzyme PDE did not fascinate as much attention. However, it has long been known that in most cells and tissues the maximal catalytic capacity for cAMP synthesis is approximately ten times less than the capacity for hydrolysis by PDEs and one of the key factors determining turnover of cAMP (Barber et al. 1992). This feature is consistent with general observations that even partial inhibition of PDE results in a many-fold increase in the accumulation of cAMP and the activation of protein kinase, signaling pathway (Houslay and Milligan 1997). Furthermore, it increasingly evident that PDE activities serve more than one function. PDE activity may not only determine the amplitude of cyclic nucleotide-mediated hormonal responses, but also modulates the duration of the signal and in some systems they also modulate rapid oscillation of cyclic nucleotides, as exemplified in regulation of PDE's function (Beavo 1995; Houslay and Milligan 1997).

Recent advances in molecular genetics and biochemistry have revealed that vertebrate PDEs constitute a large superfamily of related isozymes, comprising several gene families (types), single gene products (subtypes), and numerous isoforms-splice variants (Beavo et al. 1994). The systematic classification of the PDE isozyme system is based primarily on cDNA genetic codes and primary amino acid sequences, which can virtually unequivocally identify any mammalian PDE isozyme.

Informations on PDEs in subdivision of the nephron are obtained from the studies of isolated glomeruli, suspension of tubules, and microdissected nephron segments. The initial study of rat glomeruli showed that activities of cGMP PDE are higher than cAMP-PDE and that cGMP stimulates cAMP-PDE (Torres et al. 1978). Recent studies using selective PDE inhibitors indicate the presence of PDE2 in high activity, with lower activities of PDE3 and PDE4 (Matousovic et al. 1997). In glomeruli, cGMP is hydrolyzed by two isozymes; PDE5 is more abundant than PDE1. Localization of PDE isozymes within the three major cell types that populate glomeruli is not yet known or is only suspected by comparison with PDE isozyme profiles in glomerular cells grown in culture (Matousovic et al. 1995). Knowledge of PDE isozyme patterns in glomeruli provides useful basic information for studies of pathophysiologic changes in cyclic nucleotide signal transduction systems. In glomeruli from nephrotic rats, it was found that increased cGMP PDE activity and PDE5 inhibitor zaprinast would correcte the defect in cGMP accumula-

tion (Valentin et al. 1992). Although the type of cells containing increased cGMP PDE remains to be determined, analysis of the cGMP responses to atrial natriuretic peptide (ANP) in whole glomeruli already points to increased PDE5 activity as a pathogenic factor.

PDE isozymes in extracts of tubular suspensions from cortex show a substantially different profile than from glomeruli (Matousovic et al. 1997). PDE4 has the highest activity, while PDE3 and PDE1 are much less active; all of these PDEs hydrolyze cAMP. In contrast to glomeruli, where PDE2 is not detectable, cGMP is hydrolyzed equally by PDE1 and PDE5. More nephron segemnt-specific information was obtained from measuring cAMP PDE and cGMP PDE activities in microdissected segments of tubules in the studies by Jackson et al. (1980b). Most information about tubular PDEs has accumulated from studies of segemnts of collecting ducts; however, virtually all were conducted without using a selective PDE inhibitor or modulator (Dousa 1999). Results of these studies show that cGMP PDE activity is rather higher than cAMP PDE activity in the OMCD, inner medullary collecting duct (IMCD) and mTAL. Data also indicates the presence of PDE1 in mTAL and in OMCD. Study with the use of selective PDE inhibitors indicates the presence of PDE4 and PDE3 in IMCD. Stimulation of protein kinase C (PKC) increased cAMP PDE, apparently PDE4, in OMCD but not in mTAL or PST of the rat nephron (Tetsuka et al. 1995). Observations based on indirect evidence reported by another group suggest that PKC activation may increase PDE1 activity in rabbit proximal tubular cells (LeGoas et al. 1991). Besides our current study, distribution of the PDE isozyme in defined nephron segments remains to be examined with current, refined methods. The availability of selective PDE isozymes, as well as the possibility of determining mRNA encoding various PDE proteins by reverse transcriptase polymerase chain reaction, by in situ hybridization, and by immunohistochemical methods should make the study of PDE isozyme systems in specific nephron segments a rewarding subject of future investigations.

As for the role of cAMP PDE in the renal physiology and pathophysiology, the exsistence of enzymatic activity that catalyzes hydrolysis of cAMP was noted in earlier studies when cAMP system was investigated in many organs that were well known target organs for hormone action (Dousa and Rychlik 1968). Subseqently, role of PDE in renal physiology was examined with the use of whole kidney preparation (Thompson et al. 1979). However, in the investigation of adenylate cyclase that are regulated by hormones, neurotransmitters and autacoids attracted much more attention than the investigation of PDEs. Consequently, studies for PDE in the kidney are relatively few. Since informations about PDE from whole kidney preparations were limited, it become obvious that expression and functional significance of PDE isozymes ought to be investigated in specific nephron segements. In the late 1970's and early 1980's did the nephron heterogeneity become recognized in terms of the regulation by hormones that act through cAMP and signaling (Morel et al. 1981). The concept of a superfamily of well-defined PDE isozymes emerged in the late 1980's, and "second-generation" PDE isozyme inhibitors become available at that time (Beavo and Reifsnyder 1990; Manganiello et al. 1995). Therefore, there are a few studies to examine the functions of PDE isozymes or isoforms in specific nephron segments to date. This development may be illustrated by the studies of the cAMP-mediated signaling system in the antidiuretic response to vasopressin (AVP) in a murine model of nephrogenic diabetes insipidus (NDI), the so called NDI mice (Dousa and Valtin 1974; Jackson et al. 1980a; Takeda et al. 1991). In the first study of NDI mice, involvement of PDE was neither detected nor suspected (Dousa

and Valtin 1974). Later investigations on microdissected segements of collecting ducts established that increased PDE activity plays a dominant role to be resiatant to exogenous AVP (Jackson et al. 1980a), and an even later study with the use of selective PDE inhibitors pointed to the abnormally high activity of PDE4 in collecting ducts as a key pathogenic factor (Takeda et al. 1991). In addition, evidence to date suggests that blunted response of collecting ducts to vasopressin in adrenal insufficiency may be due to increased activity of cAMP PDE and treatment with glucocorticoid could correct the defect by diminishing cAMP-PDE activity (Jackson et al. 1983).

Based on these facts, it is quite important to know the segmental activity of cAMP PDE activity to gain a better understanding of a ceratain type of renal disease. Therefore, results of studies reported here brings the first basic information about activity of cAMP catabolizing enzymes in three mammalian species which are frequently used as models in renal physiology and pathophysiology. As suggested, results of studies on cAMP PDE in glomeruli and tubules isolated from rat renal cortex, cAMP PDE activity in tubules was considerably higher than in glomeruli (Torres et al. 1978). However, cortical tubular suspension contain various cortical segments, namely PCT, PST, cTAL, DCT, CNT and CCD. Therefore, we can't conclude that all cortical segments have higher cAMP PDE activities than glomeruli.

As shown in our present results, each tubular segment had different cAMP PDE activity. When cAMP PDE activity were expressed per mm tubular length or per one glomerulus, all tubular segments showed higher enzyme activities than glomerulus. However, this may not be the case when enzyme activity was expressed per unit protein content in PCT and glomeruli, namely in all three species, glomeruli showed rather higher enzyme activity than PCT. So we can assume that higher cAMP PDE activity per unit protein content in cortical tubular suspension could be due to the high enzyme activities of other nephron segments rather than PCT. Namely, as shown in our results, cAMP PDE activity in tubular segments distal to the loop of Henle, with the exception of cTAL and DCT of rabbit kidney, had higher enzyme activities than in the proximal segments. Cole et al. also confirmed by the different method from us that distal segments had much higher cAMP PDE activities than proximal segments (Cole et al. 1982). Functional significance of this finding is not clear, but several factors, cAMP system in tubules may be considered. Tubule segments such as cTAL, DCT and CNT are, according to a review by Morel, endowed with adenylate cyclase which is sensitive to stimulation of several potent hormones (Morel 1981). On the other hand, proximal tubules (both PCT and PST) are segments of adenylate cyclase sensitive to stimulation, virtually by a single hormones, namely PTH. It is thus conceivable that in mouse and rat kidney relative high activity of cAMP catabolizing enzyme in cTAL, DCT and CNT is required for intense catabolism of cAMP generated in response to several stimulatory inputs. In fact, these nephron segments generate cAMP formation by many hormonal stimuli, namely PTH, VP, calcitonin, glucagon and β-adrenergic agonists. However, this may not be the case of cTAL and DCT from rabbit kidney. In this specie, these two nephron segments possess only few hormone sensitive adenylate cyclase. This is quite different feature compared to the rat kidney. cTAL respond to PTH and calcitonin (CT) slightly and DCT respond only to CT. Therefore, it could be deduced that low cAMP PDE in cTAL and DCT from rabbit kidney is good agreement with few hormone sensitive adenylate cyclase distributions in these nephron segments. In general, the intense of cAMP PDE activity along the nephron segments seem roughly to correlate with the distributions of hormone sensitive adenylate cyclase and/or

cAMP formation. There could be seen such a rule that the more hormone sensitive adenylate cyclase, the higher the cAMP PDE activity in each nephron segment.

Overall, the highest cAMP PDE activities were measured in mouse and the lowest measured in rabbit nephrons with those of the rat nephrons occupying an intermediate position (Fig. 1). These species differences were also confirmed in another kidney enzyme (e.g. Na, K, ATPase) where the highest in rat nephrons, lowest in rabbit and intermediate in mouse nephrons (Katz et al. 1979). Relatively high activity of cAMP PDE in distal nephron segments may relate to findings on renal response to a variety of hormones in terms of excretion of nephrogenic cAMP. As a rule, the ones acting on proximal segments (PTH) result in an increase in nephrogenic cAMP, while hormones acting distally (vasopressin, glucagon, epinephrine) does not cause a consistent increase in nephrogenic cAMP. It was proposed that the lack of increase of nephrogenic cAMP in response to hormone acting on distal segments is due to the permeability of distal luminal membrane to cAMP. The finding of relative high activity of cAMP PDE in distal nephron segments may suggest that cAMP generated in responses to hormones is more rapidly catabolized in distal segments and cannot reach tubule lumen in the urinary space.

Certain tricyclic antipsychotic agents were reported to inhibit calmodulin dependent cAMP PDE (PDE1) activity in various tissues (Prozileck and Weiss 1982; Takaichi and Kurokawa 1988). In this report, we examined the effect of one of these tricyclic agents, trifluoperazine, which was believed to be a specific inhibitor of PDE1. In preliminary experiment by using mixed cortical tubules from rat kidney, we confirmed that maximum inhibition was obtained at the concentration of 200 μM of TFP. In subsequent experiments of isolated nephron segments, this concentration of TFP showed inhibition in all segments of the tubules tested in different extent. Although there are some trends that higher degree of inhibition was observed in G, CNT, OMCD and the lesser degree of inhibition was found in PCT and DCT, there is no statistical significance of its inhibition among nephron segments. Therefore, these findings suggest that virtually all nephron segments are equally dependent on trifluoperazine-sensitive pathway that may reflect the Ca^{2+}-calmodulin system. In another words, the proportion of PDE1 in total cAMP PDE activity may not differ in each nephron segment. Since the present study examines only basal cAMP PDE activity without stimulation by hormones or neurotransmitters, it seems to be interesting to measure the cAMP PDE activity with hormones or neurotransmitters which stimulate the increase of intracellular Ca and see the change of proportion of PDE1 in total cAMP PDE activity. Although we only investigated the effect of TFP as an inhibitor of Ca^{2+}-calmodulin on cAMP PDE in the present study, other Ca^{2+}-calmodulin inhibitors should be examined in such experiments to verify the dynamic change of PDE1.

High concentrations of ATP and pyrophosphate, the substrate and product of adenylate cyclase, were reported to inhibit cAMP PDE in various tissues including the kidney (Dousa and Rychlik 1970). It was suggested that inhibition by high concentration of ATP could be the results of the chelation of metal ions in the enzyme incubation mixture. As shown in our current results, cAMP PDE inhibition by ATP in microdissected OMCD and mTAL occurred in the concentration range from 5 mM to 10 mM of ATP. These results are good agreement with previous reports by us and others where mM range of ATP concentrations might inhibit cAMP PDE activity (Cheung 1966; Dousa and Rychlik 1970). However, it was calculated that OMCD and mTAL possessed about 0.17 mM and 0.10 mM cellular ATP concentrations, respectively. Therefore, these ranges of ATP concentrations are not sufficient to inhibit

cAMP PDE, since actual inhibition of cAMP PDE by ATP requires over 5 mM ATP concentrations that may never reach in a normal epithelial cell.

In summary, the present study shows that distal segments of tubules have more active catabolism of cAMP than proximal segments. The proportion of PDE1 in basal cAMP PDE activity may not differ in each nephron segment. Cellular concentration of ATP might not be involved in the regulation of the total low Km cAMP PDE activity in rat mTAL and OMCD.

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