Compartmentalization of cAMP Signaling in Mesangial Cells by Phosphodiesterase Isozymes PDE3 and PDE4

REGULATION OF SUPEROXIDATION AND MITOGENESIS

Claudia C. S. Chini, Joseph P. Grande‡, Eduardo N. Chini§, and Thomas P. Dousa¶

From the Renal Pathophysiology Laboratory, Department of Physiology and Biophysics, and the Department of Laboratory Medicine, Mayo Clinic and Foundation, Mayo Medical School, Rochester, Minnesota 55905

Some major pathobiologic processes in renal mesangial cells, elicited in response to immunoinflammatory stimuli, are modulated via cAMP-protein kinase A (PKA) signaling pathways; namely, generation of reactive oxygen metabolites (ROM) and accelerated proliferation of mesangial cells. We investigated the role of cAMP phosphodiesterase (PDE) isozymes in these regulatory mechanisms. Generation of ROM in cultured rat mesangial cells was inhibited by selective inhibitors of PDE4, rolipram and denbufylline, whereas PDE3 inhibitors, cilostamide or lixazinone, had no effect. Conversely, cilostamide or lixazinone suppressed mitogenic synthesis of DNA in mesangial cells, but 1 μM rolipram or 1 μM denbufylline showed no inhibitory effect. The efficacy of PDE isozyme inhibitors (IC \(_{50}\)) to suppress \[^{3}H\]thymidine incorporation or ROM generation paralleled IC \(_{50}\) values for inhibition of cAMP PDE. Incubation of mesangial cells with either rolipram alone or with cilostamide alone increased significantly in situ activity of PKA in mesangial cells, assessed by (−cAMP/+cAMP) PKA activity ratio, and the stimulatory effects were additive. Results indicate that in mesangial cells a cAMP pool that is metabolized by PDE4 activates PKA and thereby inhibits ROM generation; another cAMP pool that is metabolized by PDE3 activates another PKA (isozyme or pool) which suppresses proliferation of mesangial cells. We propose that in mesangial cells, a cAMP-PKA pathway that regulates mitogenesis is determined by activity of PDE3, whereas another cAMP-PKA pathway is directed by activity of PDE4 and controls ROM generation. Therefore, two PDE isozymes within one cell type compartmentalize distinct cAMP signaling pathways.

Mesangial cells in kidney glomeruli are specialized pericytes (1) that are located in the intercapillary spaces of glomeruli and comprise about 30% of glomerular cells (2). Mesangial cells have some properties, such as contractility, reminiscent of smooth muscle cells, and some that are common to monocytes and macrophages, i.e. phagocytosis or the ability to generate reactive oxygen metabolites (ROM)\(^3\) (3, 4). According to the current view, mesangial cells have a key role in maintaining the integrity of renal glomerular structure and function mainly by regulating capillary blood flow, uptake of macromolecules by phagocytosis, and synthesis of the extracellular matrix (1, 3, 4). Pathobiologic responses of mesangial cells to immunoinflammatory stimuli often determine and/or contribute to pathogenic processes involved in the development of acute or chronic glomerulonephritides, in particular, mesangial proliferative glomerulonephritis (5, 6).

In our recent studies of rat mesangial cells grown in primary culture we observed that inhibition of cyclic-3′,5′-nucleotide phosphodiesterase (PDE) isozyme type-3 (PDE3) by selective inhibitors causes activation of cAMP-protein kinase A (PKA) and suppresses mesangial cell proliferation, either basal or stimulated by addition of growth factors such as epidermal growth factor or platelet-derived growth factor (7). Inhibitors of PDE isozyme type-4 (PDE4) had only minor or no inhibitory effects upon mesangial cell mitogenesis (7). In rats with experimentally induced mesangial proliferative glomerulonephritis, administration of a PDE3 inhibitor alone (8) or with a PDE4 inhibitor (9) suppresses development of glomerulonephritis, including decreased proliferation of mesangial cells (8, 9). We also observed that generation of ROM in freshly isolated rat glomeruli is suppressed when the cAMP-PKA pathway is activated by incubating whole glomeruli with rolipram, a selective inhibitor of PDE4, and to a lesser degree with cilostamide, a selective inhibitor of PDE3 (10). Interpretation of these results was limited since all three cell types populating renal glomeruli, i.e. mesangial, epithelial, and endothelial cells, are endowed with NADPH oxidase, an enzyme complex that catalyzes ROM generation (11, 12). Therefore, all of these cell types can contribute to ROM production by the whole glomeruli; it remained unknown in which cells and to what extent inhibitors of PDE3 and/or PDE4s exert its inhibitory effects upon ROM generation (10). Further, it cannot be excluded that some of blood cells trapped within glomeruli, namely neutrophils that have very active NADPH oxidase (13), contribute to glomerular ROM generation. Studies of several types of leukocytes, e.g. eosinophils (14), monocytes (15), and neutrophils (16), all highly active in generation of ROM, have shown that only PDE4 inhibitors suppress NADPH oxidase and ROM synthesis in these cell types (14–16).

Extracts of rat mesangial cells grown in primary culture (7) have the capacity to catalyze hydrolysis of cAMP by PDE3 and,\(^1\)

\(^{1}\)The abbreviations used are: ROM, reactive oxygen metabolite(s); PDE, phosphodiesterase; PKA, protein kinase A; DCFH, 2′,7′-dichlorodihydrofluorescein; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; SOZ, serum-opsonized zymosan; MES, 4-morpholinoethanesulfonic acid.

\(^3\)The study was supported in part by Grants DK-16105 (to T. P. D.) and DK-45280 (to J. P. G.) from the NIDDK, National Institutes of Health, by a grant-in-aid from the American Heart Association, Minnesota Affiliate, and by the Mayo Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\(^{¶}\) Recipient of a research fellowship from the National Kidney Foundation Inc.

\(^{‡}\) To whom correspondence should be addressed: Mayo Clinic, 921B Guggenheim Bldg., 200 First St., S.W., Rochester, MN 55905. Tel.: 507-284-4343; Fax: 507-266-4710.
to a significantly higher degree, by PDE4. These are the two major PDE isozymes with low \( K_m \) for cAMP present in rat mesangial cells (7). The question arises as to which of these two PDE isozymes (or both), and to what degree, metabolizes the presumed cAMP pool that specifically modulates ROM generation in mesangial cells; we set out to investigate this problem. We also addressed a more general and largely unsolved question, i.e. whether within one cell type, in this instance mesangial cells, two distinct cellular functions that are both known to be modulated by cAMP-PKA signaling pathways are under separate control of two distinct cellular pools of cAMP which are functionally linked to, and thereby compartmentalized by, activity of specific PDE isozymes. The findings reported herein support a hypothesis that in rat mesangial cells, the ROM generation catalyzed by NADPH oxidase is regulated by a cAMP pool functionally coupled to isozyme PDE4, whereas regulation of mitogenesis in mesangial cells is regulated by another cAMP pool that is regulated by activity of PDE3. The results thus provide evidence for the intracellular compartmentalization of cAMP signaling pathways effected by PDE isozymes within mesangial cells.

EXPERIMENTAL PROCEDURES

Cell Culture—Studies were conducted on rat mesangial cells isolated from freshly prepared glomeruli and grown in primary culture as described in detail in our preceding papers (10, 17). Specifically, mesangial cells (passages 2–18) were grown in an RPMI medium with 20% fetal calf serum until reaching confluence; fetal calf serum was withdrawn for 24–48 h prior to experiments. Mesangial cells were grown on multiple well plates until confluence, prior to measurement of ROM generation (12-well plate), the rate of mitogenesis or cAMP accumulation (24-well plate), or measurement of [in situ] PKA activity (6-well plate), as in our preceding reports (10, 17).

Rate of ROM Generation—This was determined with the probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), in a microfluorometric assay originally designed for measurement of the ROM burst in monocytes (18). The same probe was also employed for detection of ROM in cultured vascular smooth muscle cells (19). DCFH-DA is a compound that is membrane-permeant because of two esterified acetates, which are cleaved by esterases in the cells (14). Both DCFH-DA and the deesterified product 2',7'-dichlorodihydrofluorescein (DCFH) are nonfluorescent, but both are readily oxidized by ROM to a fluorescent 2',7'-dichlorofluorescein derivatives (18, 19). This method detects both ROM that remain within the cell and ROM in the extracellular medium (18, 19). For measurement of ROM generation, mesangial cells were incubated with additions, including the probe, as specified. The development of fluorescence initiated by the added stimulus was expressed as relative fluorescence units monitored at 485 nm excitation and at 530 nm emission wavelength in microplate fluorometer interfaced with a PC-compatible computer for data processing (18).

ROM production was determined in mesangial cells stimulated with serum-sonopsonized zymosan (SOZ) (zymosan opsonized by incubation with fresh rat serum) added at a final concentration of 2 mg/ml. It has been shown that SOZ stimulates both ROM generation and phagocytosis in mesangial cells (20). The development of SOZ-stimulated fluorescence was almost completely blocked (94 ± 2%; \( n = 10 \)) by the addition of superoxide dismutase (40 \( \mu \)g/ml) and also was suppressed by other scavengers of ROM, i.e. catalase and N-acetylcysteine (data not shown). The difference in fluorescence without and with superoxide dismutase (Fig. 1), "the superoxide dismutase-suppressible value," was taken as a measure of ROM generation. In control experiments, we determined that none of the test compounds employed in our study either quenched fluorescence or emitted spurious fluorescence at the detection wavelength under the experimental conditions. In preliminary experiments, this method produced similar results in assessing ROM generation in the murine macrophage cell line J774 (18). The superoxide dismutase-suppressible fluorescence generated by SOZ-stimulated mesangial cells accumulated linearly with time for at least 2–3 h (Fig. 1). Inhibition by 10 \( \mu \)M rolipram (35%) or 10 \( \mu \)M forskolin (33%) was the same at 1, 2, or 3 h of incubation. Therefore, fluorescence readings were made after a 3-h incubation to optimize precision of measurements.

In Situ Activation of PKA—PKA activation was assayed by the method described by Corbin et al. (21), with minor modifications (7, 10). Quiescent mesangial cell cultures in Petri dishes (6-cm diameter) were incubated either without additions (controls), with PDE inhibitors for 60 min, or with forskolin for 10 min. Incubations were terminated by chilling plates (0–4 °C) and quickly scraping cells, which were then pelleted by centrifugation at 2,500 \( \times \) g for 5 min at 0–4 °C. The supernatant was discarded, and mesangial cells were homogenized in buffer containing (final concentration) 0.25M sucrose, 0.5 mM 3-isobutyl-1-methylxanthine, 4 mM EDTA, and 20 mM MES-NaOH, pH 6.8. The homogenate was centrifuged at 27,000 \( \times \) g for 30 min at 0–4 °C, protein content was determined, and PKA activity was assayed in the supernatant using Leu-Arg-Ala-Ser-Leu-Gly (Kemptide) as a substrate (7, 22). PKA activity was assayed without and with added 1 mM cAMP and 1 mM cAMP plus a maximal inhibitory concentration (3 \( \mu \)M) of a specific PKA oligopeptide pseudosubstrate inhibitor WIPTIDE (7, 10, 22). The difference between PKA activity without and with 10 \( \mu \)M WIPTIDE was taken as specific PKA activity and expressed as (−cAMP/−cAMP) PKA activity ratio (7, 10, 22).

Mitogenic Synthesis of DNA—DNA synthesis was measured in mesangial cells that were rendered quiescent by incubation in medium with 0.5% fetal calf serum for 72 h. After overnight (16 h) incubation with test agents, the cells were incubated with [\( ^{3}H \)]thymidine for 4 h, and incorporation of [\( ^{3}H \)]thymidine was determined as described previously (7, 10, 22).

Accumulation of cAMP—This was determined by incubating mesangial cells with test agents and terminating the incubation by adding 5% trichloroacetic acid (final concentration) to wells (cells were collected by scraping with a rubber policeman and the mixture incubated on ice for 30 min). Trichloroacetic acid-precipitated protein was extracted with water-saturated ether, and the cAMP content was measured by radioimmunoassay as described (7, 10).

PDE Activity—PDE activity was determined in total mesangial cell extracts prepared by homogenization in buffer containing 0.1% Triton X-100 (7, 10). Briefly, PDE activity was measured by incubating portions of homogenates in a reaction mixture (final volume 110 \( \mu \)l) containing (final concentrations) 10 mM MgSO\(_4\), 2 mM EGTA, 0.1% bovine serum albumin, 15 mM Tris-HCl adjusted to pH 7.4, and 0.5 \( \mu \)M [\( ^{3}H \)]cAMP as substrate. Hydrolysis of cAMP was less than 20% and was linearly proportional to incubation time and enzyme protein concentration (7, 10). In experiments examining the dose response to PDE inhibitors, maximal inhibition by rolipram or cilostamide was reached at 3 \( \mu \)M. Therefore, as in our preceding studies (7, 10), we employed 3 \( \mu \)M rolipram and 3 \( \mu \)M cilostamide as maximally effective inhibitory doses. The proportion of PDE3 and PDE4 activities in extracts from mesangial cells was defined as cAMP PDE activity inhibited by 3 \( \mu \)M cilostamide or 3 \( \mu \)M rolipram, respectively (7). In previous studies with cultured mesangial cells (7, 21), the proportion of PDE3 activity accounted for ~30% and PDE4 for ~60% of total PDE activity in homogenates assayed with 0.5 mM cAMP as substrate.

General Methods and Design—The effects of PDE inhibitors or other agents upon responses of cultured mesangial cells were measured during a 72-h incubation in a medium containing 0.5% fetal calf serum (7) under "ambient conditions" without added exogenous growth factors, hormones, cytokines, or agonists of adenylate cyclase, i.e. under the autocrine/paracrine influence of autacoids and other regulatory factors generated by mesangial cells themselves (5, 6). Protein measurements were done by the Lowry method (23). Stock solutions of all inhibitors were made in 100% dimethyl sulfoxide; incubation media in all conditions, including controls, contained 0.1% dimethyl sulfoxide (final concentration), which did not interfere with measured parameters. Values for IC\(_{50}\) (concentration of PDE inhibitors required to inhibit by 50% cAMP PDE activity, [\( ^{3}H \)]thymidine incorporation or ROM generation) were determined from concentration-response curves in which inhibitor concentrations ranged from 1 \( \times \) 10 \( ^{-7} \) to 10 \( \times \) 10 \( ^{-7} \). IC\(_{50}\) values are means ± S.E. from three or four experiments.

The results were evaluated statistically with the use of two-tailed Student’s t test; values of \( p < 0.05 \) were considered statistically significant. NS denotes no significant difference.

Materials—Cilostamide, \( N \)-cyclohexyl-N-methyl-4-(1,2-dihydro-2-oxo-6-quinoloxly), butyramide (OPE-36393), and cilostol, \( 6 \)-[4-1-cyclohexyl-1H-tetrazol-5-ylbutoxy]-3,4-dihydro-2-(\( \mathrm{H} \))quindoline (OPE-13013) were gifts from Otsuka Pharmaceutical Company (Osaka, Japan). Rolipram (racemic), 4-(3-cyclopropyl-4-ethylphenyl)-2-pyridolide (ZR 62711), was a gift from the Wyeth Laboratories (Cedar Knolls, N.J.). Denbufylline, BRL 30882 (3,1-di-n-butyl-7'-2'-oxoxypropyl)-xanthine, was donated by Smith Kline Beecham Pharmaceutical (Worth ing, Sussex, United Kingdom). Lixazinone, -cyclohexyl-N-methyl-4-(7-oxyl-1,2,3,5-tetrahydromidazol[2,1-b]quinazolin-2-one)-butyramide (RS-82856), as a gift from Dr. R. Alvarez, Syntex Research, Palo Alto, CA.
RESULTS

ROM generation and accumulation in cultured mesangial cells were linearly proportional to time of the incubation (Fig. 1). Incubation with dibutyryl cAMP, forskolin, or rolipram, a selective PDE4 inhibitor, all resulted in marked inhibition (30–40%) of ROM generation, and the extent of inhibition was not significantly different among the three agents (Fig. 2). The inhibitory effect of rolipram was dose-dependent with maximal inhibition at 1 μM (Fig. 3 and Table I). Denbufylline, an inhibitor of PDE4 which is structurally unlike rolipram (24), inhibited ROM generation to a degree similar to that of rolipram (Table I). In contrast, under the same experimental conditions, two structurally dissimilar selective PDE3 inhibitors, cilostamide and lixazinone (25), had no effect on ROM generation (Table I). Incubation of mesangial cells with 10 μM rolipram and 10 μM cilostamide together did not inhibit ROM generation (45 ± 6%, mean ± S.E.; n = 7) to a significantly greater extent than 10 μM rolipram alone (32 ± 4%, mean ± S.E.; n = 7) (Fig. 2, lower panel). The IC50 values for rolipram inhibition of cAMP PDE activity and ROM generation were similar (Table II).

The effects of the PDE4 inhibitors, rolipram and denbufylline, and the PDE3 inhibitors, cilostamide and lixazinone, on mitogenesis in mesangial cells were opposite to their effects upon ROM generation. Incubation with 3 μM cilostamide inhibited [3H]thymidine incorporation into mesangial cells (67 ± 3%, mean ± S.E.; n = 7) four times greater (p < 0.005; t test) than that caused by 3 μM rolipram (17 ± 4%, mean ± S.E.; n = 7) or 3 μM denbufylline (14 ± 4%, mean ± S.E.; n = 7). At lower (1 μM) concentrations of PDE inhibitors, differential selective inhibition of ROM generation and mitogenesis was even more clear-cut. Both 1 μM cilostamide and 1 μM lixazinone suppressed (>42%) markedly [3H]thymidine incorporation, whereas 1 μM rolipram and 1 μM denbufylline were ineffective (Table I). Further, we found that IC50 values for inhibition of cAMP PDE by cilostamide and lixazinone correspond closely to their IC50 values for suppression of [3H]thymidine incorporation (Table II); the IC50 values for lixazinone were, for both parameters, about 2 orders lower than IC50 for cilostamide (Table II).

The effects of incubation of mesangial cells with PDE isozyme inhibitors and forskolin upon in situ activation of PDE4 in mesangial cells, as determined by measuring (−cAMP/+cAMP) PKA activity ratios, and upon inhibition of ROM generation in mesangial cells are depicted in Fig. 2. Incubation with either rolipram alone or cilostamide alone increased significantly (and to a similar extent) the in situ PKA activity in mesangial cells (Fig. 2). However, cilostamide exhibited no effect upon ROM, whereas rolipram caused maximum inhibition of ROM generation (Fig. 2). The increase in in situ PKA activation in response to incubation of mesangial cells with 10 μM rolipram and 10 μM cilostamide added together (Δ = 338 ± 42; mean ± S.E., n = 9), expressed as the increment in (−cAMP/+cAMP) PKA ratio, was equal to or higher than the arithmetic sum of PKA responses to 10 μM rolipram added alone (Δ% = 97 ± 32; mean ± S.E., n = 9) and to 10 μM cilostamide added alone (Δ% = 129 ± 42; mean ± S.E., n = 9). Yet, the extent (−Δ%) of inhibition of ROM generation either...
by rolipram alone, by rolipram and cilostamide added together, or by forskolin was not significantly different (Fig. 2). Interestingly, incubation with rolipram alone or cilostamide alone resulted in in situ PKA activation (Fig. 2) without any measurable increase in cAMP content after incubation for 1 h (7) or 16 h. At the time of pulse labeling with $[^3H]$thymidine (16 h), the cAMP content (in pmol/well ± S.E.) was 1.23 ± 0.04 (controls), 1.17 ± 0.76 (with 10 μM rolipram), 1.11 ± 0.72 (with 10 μM cilostamide), and 15.7 ± 3.5 (with 10 μM forskolin).

The effects of PDE4 inhibitors or PDE3 inhibitors on cAMP accumulation in mesangial cells were measured when cAMP synthesis was stimulated by forskolin, a potent direct stimulator of adenylate cyclase. Under these conditions PDE4 inhibitors, denbufylline and Ro-20-1714, caused a 200-fold increase in cAMP accumulation in mesangial cells (Fig. 4). In contrast, PDE3 inhibitors lixazinone and cilostazol caused only minor elevation of cAMP (Fig. 4).

**DISCUSSION**

The observations presented herein argue in support of the hypothesis that PDE isozymes can, within a single cell type (i.e. mesangial cell), direct or compartmentalize the cAMP-PKA signaling pathways that regulate two distinct cellular functions. The results of the present study conducted on homogenous populations of mesangial cells grown in vitro indicate that generation of ROM in mesangial cells is regulated by a cAMP-PKA pathway that is uniquely linked to activity of PDE4 isozymes and, conversely, that mitogenesis in mesangial cells is regulated by a cAMP-PKA pathway specifically linked to activity of PDE3 isozymes (Figs. 2 and 3 and Table I).

The existence in mesangial cells of at least two functional and/or ultrastructural compartments of cAMP and related PKA which are determined by activities of PDE3 and PDE4, respectively, is supported by several lines of evidence. First, two structurally dissimilar inhibitors of PDE4 (rolipram and denbufylline) suppressed ROM generation, whereas two structurally dissimilar inhibitors of PDE3 (lixazinone and cilostamide) had no inhibitory effect (Table I). Conversely, 1 μM cilostamide or lixazinone, but not 1 μM rolipram or 1 μM denbufylline, inhibited $[^3H]$thymidine incorporation in mesangial cells (Table I). Second, the efficacy of selective PDE isozyme inhibitors (IC$_{50}$) for inhibition of cAMP PDE closely corresponded to their respective potencies (IC$_{50}$) for suppression of either ROM generation or mitogenesis (Table II).

Third, the existence of PDE isozyme-determined cAMP-PKA compartments in mesangial cells is indicated by observations on in situ activation of PKA (Fig. 2). As documented by others (21) and our previous studies (7, 22), measurements of the in situ activity of PKA, determined as (−cAMP/cAMP) PKA activity ratios (7, 21) or by similar methods (25, 26), are much more sensitive indices of the in situ activation of cAMP-PKA signaling pathways than is determination of the total cellular (or tissue) content of cAMP (7, 26, 27). Results of our experiments examining in situ PKA activation in mesangial cells (Fig. 2) argue for the existence of two separate PKAs (PKA isozymes or pools) which are independently activated by cAMP that is increased by selective inhibition of PDE3 or PDE4, respectively. Although incubation with either rolipram alone or cilostamide alone resulted in an increase of in situ PKA activity (Fig. 2 and “Results”), only PKA activation elicited by rolipram, but not cilostamide, was associated with decreased ROM generation in mesangial cells (Fig. 2). In mesangial cells incubated with rolipram and cilostamide together, the extent of in situ PKA activation was at least additive, and perhaps even synergistic (Fig. 2; see also “Results”); yet, the inhibition of ROM generation by rolipram and cilostamide combined was not significantly greater than by rolipram alone (Fig. 2). Therefore, it appears that the portion of mesangial cell PKA which is activated in response to cilostamide has no functional relationship to inhibition of ROM generation (Fig. 2). Conversely, the inhibition of mitogenesis in mesangial cells by cilostamide alone

---

**TABLE I**

Comparison of the effects in mesangial cells of PDE4 antagonists, rolipram and denbufylline, and PDE3 antagonists, cilostamide and lixazinone, upon DNA synthesis and ROM generation, measured as described under “Experimental Procedures.” The effects are expressed in relative terms as Δ% difference from basal values (without addition of test compound) taken as 100%

|          | $[^3H]$Thymidine incorporation Δ% | p value | ROM generation Δ% |
|----------|-----------------------------------|---------|--------------------|
| **Denbufylline** |                                    |         |                    |
| 1 μM     | +5 ± 4                             | NS      | Rolipram 10 μM     |
| 10 μM    | +4 ± 18                            | <0.02   | 1 μM               |
| **Lixazinone** |                                    |         |                    |
| 1 μM     | −46 ± 2                            | <0.01   | Denbufylline 1 μM  |
| 10 μM    | −42 ± 8                            | <0.05   | 10 μM              |
| **Cilostamide** |                                    |         |                    |
| 1 μM     | −70 ± 7                            | <0.01   | Lixazinone 10 μM   |
| 10 μM    | −70 ± 7                            | <0.01   |                    |

* Each value denotes mean ± S.E. of three experiments.

* p values for statistical significance of changes (±Δ%) by paired t test. NS, not significant.

* Significantly different (p < 0.05, t test) from value with 10 μM rolipram.

* Not significantly different from values with 10 μM rolipram and 1 μM or 10 μM denbufylline.
was not significantly different from the effect of cilostamide and rolipram combined, whereas the in situ activation of PKA in response to cilostamide and rolipram added together was significantly higher than PKA activity elicited by cilostamide alone or rolipram alone (Fig. 2; see “Results”). Taken together, the additivity of in situ PKA activation in response to incubation with rolipram and cilostamide, both at maximally effective concentrations, suggests that increases of intracellular cAMP content in mesangial cells elicited by inhibition of PDE4 or that of PDE3 do activate different portions of cellular PKA. Conceivably, the two cAMP pools regulated by different PDE isozymes may activate two distinct PKA isozymes, i.e. PKA-I and PKA-II (28), or alternatively, the same PKA type (e.g. PKA-II) which within the same cell is attached via specific anchoring proteins to different cellular ultrastructures (29, 30). The exact sequence of interactions between cAMP pools determined by PDE isozymes PDE3 and PDE4, related PKA isoforms, and the functional/structural targets within the mesangial cells remains to be elucidated. However, the present experimental evidence indicates that cAMP processing by specific PDE isozymes is a determining step that targets cAMP signaling pathways to specific cell functions.

At higher molar concentrations (≥3 μM) PDE4 inhibitors caused minor but distinct suppression of mitogenesis, although at lower concentrations (1 μM), neither rolipram nor denbufylline inhibited mitogenesis (Table I and “Results”). On the other hand, PDE3 inhibitors are potent blockers of proliferation (Tables I and II) but even at 10 μM had no effect upon ROM generation (Table I). The cause of this phenomenon is not yet clarified, but the following interpretation should be considered. When mesangial cells were incubated with the direct stimulator of adenylate cyclase, forskolin, the addition of PDE4 inhibitors, denbufylline and Ro 20-1714, resulted in a huge (200 times) increase in cAMP, whereas addition of PDE3 antagonists, lixazinone and cilostazol, merely doubled cAMP content (Fig. 4). These findings, which are in accord with previously observed effects of rolipram and cilostamide (7), strongly suggest that the intracellular pool of cAMP which is metabolized by PDE4 in mesangial cells is considerably larger than the pool metabolized by PDE3. Consequently, some of the cAMP accumulated in the presence of 3–10 μM selective PDE4 inhibitors might have “spilled over” into the compartment of cAMP which is specifically linked to PDE3, thereby causing a minor suppression of mitogenesis in mesangial cells (Table I). It should be stressed that at lower (1 μM) concentrations at which PDE isozyme inhibitors have the highest discriminatory power (31, 32), neither rolipram nor denbufylline had any inhibitory effect upon mitogenesis while having a maximal suppressant effect upon ROM (Table I and Fig. 3).

Although the evidence presented herein is mostly correlative and indirect, it constitutes a sound basis for the working hypothesis that in rat mesangial cells the cAMP-PKA signaling pathway that controls ROM generation is coupled to cAMP metabolism by PDE4, whereas the cAMP-PKA signaling pathway coupled to cAMP metabolized by PDE3 regulates mitogenesis. In general terms, our findings support the proposition that PDE isozymes can compartmentalize and direct cAMP-mediated cell responses toward specific functions within the same cell (33–35). Such a novel function of PDE isozymes is particularly relevant in view that activities of both PDE3 and PDE4 are highly regulated (36, 37). Activity of PDE3 isozymes can be inhibited by cGMP, activated via phosphorylation by PKA or by insulin-stimulated protein kinase (38). Likewise, some isoforms of PDE4 are up-regulated via cAMP (37); cAMP-PKA phosphorylation increases activity of the “long” isoforms such as PDE4D3 (37, 39), or cAMP can induce transcription of cognate mRNA and de novo synthesis of “short” isoforms, such as PDE4D1 (37).

Finally, considering the central role of mesangial cells in the response of glomeruli to various pathogenic stimuli (4–6), it is of potential importance that inhibitors of PDE3 and PDE4, which are effective both in vitro (7) and in vivo (8, 9), can independently suppress two major pathobiologic processes within mesangial cells. Based on this paradigm, novel “signal transduction” pharmacotherapies (40) of glomerulonephritis targeted to specific PDE isozymes may be designed and developed.

Acknowledgments—We thank Michael Thompson, Henry Walker, and Deborah C. Melder for expert technical assistance; and Carol A. Davidson and Mary E. Bennett for excellent secretarial assistance.

REFERENCES
1. Schindorff, D. (1987) FASEB J. 1, 272–281
2. Mené, P., Simonson, M. S., and Dunn, M. J. (1989) Physiol. Rev. 69, 1347–1424
3. Latta, H. (1992) J. Am. Soc. Nephrol. 3, 865–873
4. Kassgarian, M., and Sterzel, R. B. (1992) Kidney Int. 41, 524–529
5. Johnson, R. J. (1994) Kidney Int. 45, 1769–1782
6. Abboud, H. E. (1995) Kidney Int. 43, 252–267

![Figure 4](http://www.jbc.org/Downloaded from http://www.jbc.org)
Compartmentalization of cAMP Signaling

9859

7. Matousovic, K., Grande, J. P., Chini, C. C. S., Chini, E. N., and Dousa, T. P. (1995) J. Clin. Invest. 96, 401–410
8. Tsou, Y., Shankland, S. J., Grande, J. P., Walker, H. J., Johnson, R. J., and Dousa, T. P. (1996) J. Am. Soc. Nephrol. 7, 1724
9. Tsou, Y., Shankland, S. J., Grande, J. P., Walker, H. J., Johnson, R. J., and Dousa, T. P. (1996) J. Clin. Invest. 96, 262–270
10. Chini, C. C. S., Chini, E. N., Williams, J. M., Matousovic, K., and Dousa, T. P. (1994) Kidney Int. 46, 28–36
11. Neale, T. J., Ullrich, R., Ojha, P., Poczewski, H., Verhoeven, A. J., and Kerjaschki, D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3645–3649
12. Radeke, H. H., Cross, A. R., Hancock, J. T., Jones, O. T. G., Nakamura, M., Kaever, V., and Resch, K. (1991) J. Biol. Chem. 266, 21025–21029
13. Rotrosen, D. (1992) Inflammation: Basic Principles and Clinical Correlates, 2nd Ed., pp. 589–601, Raven Press, New York
14. Souness, J. E., Carter, C. M., Dioee, B. K., Hassal, G. A., Wood, L. J., and Turner, N. C. (1991) Biochem. Pharmacol. 42, 937–945
15. Turner, N. C., Wood, L. J., Burns, F. M., Gueremy, T., and Souness, J. E. (1993) Br. J. Pharmacol. 108, 876–883
16. Wright, C. D., Kuipers, P. J., Koblyara-Singer, D., Devall, I. J., Kinkefus, B. A., and Weishaar, R. E. (1990) Biochem. Pharmacol. 40, 699–707
17. Grande, J. P., Jones, M. L., Swenson, C. L., Killen, P. D., and Warren, J. S. (1994) J. Lab. Clin. Med. 124, 112–117
18. Wan, C. P., Myung, E., and Lau, H. S. (1993) J. Immunol. Methods 159, 131–158
19. Sundaresan, M., Yu, Z.-X., Ferrans, V. J., Irani, K., and Finkel, T. (1995) Science 270, 296–299
20. Ban, L., Hagege, J., Sera, J., Rondeau, E., Perez, J., and Ardaillou, R. (1983) J. Exp. Med. 158, 1836–1852
21. Corbin, J. D. (1983) Methods Enzymol. 99, 227–232
22. Chini, E. N., Chini, C. C. S., Grande, J. P., Burnett, J. C., and Dousa, T. P. (1995) Biochem. Biophys. Res. Commun. 215, 868–873
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
24. Nicholson, C. D., Jackman, S. A., and Wilke, R. (1989) Br. J. Pharmacol. 97, 889–897
25. Alvarez, R., Banerjee, G. L., Bruno, J. J., Jones, G. L., Littschwager, K., Strusberg, A. M., and Venuti, M. C. (1986) Mol. Pharmacol. 29, 554–560
26. Gapstur, S. M., Homma, S., and Dousa, T. P. (1988) Am. J. Physiol. 255, F292–F300
27. Dufau, M. L., Tsuruhara, T., Horner, K. A., Podesta, E., and Cati, K. J. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3419–3423
28. Spaulding, S. W. (1993) Endocr. Rev. 14, 632–650
29. Coghlan, V. M., Bergeson, S. E., Langeberg, L., Nilaver, G., and Scott, J. D. (1993) Mol. Cell. Biochem. 127/128, 309–319
30. Scott, J. D., and McCartney, S. (1989) Mol. Endocrinol. 18, 5–11
31. Beavo, J. A., and Reifsnider, D. H. (1990) Trends Pharmacol. Sci. 11, 150–155
32. Nicholson, R. A., Challiss, R. A. J., and Shahid, M. (1991) Trends Pharmacol. Sci. 12, 19–27
33. Elks, M. L., and Manganiello, V. C. (1984) Endocrinology 115, 1262–1268
34. Manganiello, V. C., and Elks, M. L. (1986) Mechanisms of Insulin Action, pp. 147–166
35. Schmitz-Peiffer, C., Reeves, M. L., and Denton, R. M. (1992) Cell. Signalling 4, 37–49
36. Manganiello, V. C., Murata, T., Taira, M., Belfrage, P., and Degerman, E. (1995) Arch. Biochem. Biophys. 322, 1–13
37. Conti, M., Nemoz, G., Sette, C., and Vicini, E. (1985) Endocr. Rev. 16, 370–389
38. Manganiello, V. C., Taira, M., Degerman, E., and Belfrage, P. (1995) Cell Signalling 7, 445–455
39. Alvarez, R., Sette, C., Yang, D., Eglen, R. M., Wilhelm, R., Shelton, E. R., and Conti, M. (1996) Mol. Pharmacol. 48, 616–622
40. Levitzki, A. (1996) Curr. Opin. Cell Biol. 8, 239–244
Compartmentalization of cAMP Signaling in Mesangial Cells by Phosphodiesterase Isozymes PDE3 and PDE4
REGULATION OF SUPEROXIDATION AND MITOGENESIS
Claudia C. S. Chini, Joseph P. Grande, Eduardo N. Chini and Thomas P. Dousa

J. Biol. Chem. 1997, 272:9854-9859.
doi: 10.1074/jbc.272.15.9854

Access the most updated version of this article at http://www.jbc.org/content/272/15/9854

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 38 references, 9 of which can be accessed free at http://www.jbc.org/content/272/15/9854.full.html#ref-list-1