0026-895X/93/040818-09$03.00/0

MOLECULAR PHARMACOLOGY, 44:818-826

Indirect Inhibition by Bradykinin of Cyclic AMP Generation in Isolated Rat Glomeruli and Mesangial Cells

JEAN-LOUP BASCANDS, CHRISTIANE PECHER, and JEAN-PIERRE GIROLAMI

Institut National de la Santé et de la Recherche Medicale (CJF 92.05), Institut Louis Bugnard, School of Medicine Rangueil, Toulouse 31062 Cedex, France

Received January 28, 1993; Accepted July 30, 1993

SUMMARY

The present study was designed to evaluate the effect of the activation of bradykinin (BK) receptors on intracellular cAMP levels in isolated glomeruli as well as in cultured rat mesangial cells. BK affected basal cAMP content only in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine. Furthermore, BK inhibited forskolin-, prostaglandin E_2-, and isoproterenol-stimulated cAMP accumulation, both in the presence and in the absence of isobutylmethylxanthine. The inhibitory effect of BK was independent of stimulation of cAMP degradation by phosphodiesterase. No direct inhibition of the *in vitro* adenylyl cyclase activity was observed, suggesting a requirement for cytoplasmic constituents. Use of the phospholipase A_2 inhibitor mepacrine and treatment with pertussis toxin did not modify the inhibitory effect of BK, indicating that neither the phospholipase A_2 pathway nor the inhibitory G protein is involved. The effect of BK was completely prevented by two selective protein kinase C (PKC) inhibitors, staurosporine and bisindolylmaleimide. Furthermore, use of the diacylglycerol analog 1-oleoyl-2-acetyl-*rac*-glycerol and direct activation of PKC with phorbol-12-myristate-13-acetate mimicked the effect of BK, whereas the biologically inactive phorbol ester 4α-phorbol-12,13-didecanoate was without effect. Furthermore, down-regulation of PKC by long term pretreatment with phorbol-12-myristate-13-acetate abolished the inhibitory effect of BK on stimulated cAMP levels. These results demonstrate that BK inhibits forskolin-, prostaglandin E_2-, and isoproterenol-stimulated cAMP formation through activation of the phospholipase C pathway. The subsequent production of diacylglycerol associated with stimulation of PKC in turn inhibits stimulated cAMP accumulation.

Glomeruli, and more precisely the glomerular mesangium, play a central role in the physiology and pathophysiology of renal ultrafiltration. The predominant cell type of the mesangium is the mesangial cell, which is very similar to the smooth muscle cell (1, 2). Morphological and ultrastructural studies have shown that these cells have abundant bundles of contractile filaments (3). Moreover, experiments on mesangial cells in culture have demonstrated that they respond to many vasoactive substances such as arginine-vasopressin and angiotensin II by contracting, whereas DA and PGE_2 induce relaxation (4). According to these observations, it has been suggested that the glomerular contractile mesangial cells surrounding the glomerular capillary could regulate capillary surface area. In this respect, micropuncture studies in rats support this hypothesis, because it has been demonstrated that many vasoactive agents modify the filtration process by decreasing K_f (5). K_f is the product of the total glomerular capillary filtering surface area and the hydraulic conductivity. A reduction in the value of K_f may result from a decrease of surface area, hydraulic conductivity, or both. However, because no alteration in hydraulic conductivity has been found in response to a variety of agents (6), an abundance of data indicate that a decrease in K_f results from a reduction of surface area induced by mesangial cell contraction (7). In addition, contraction of whole glomeruli by angiotensin II (8) is consistent with the presence of intraglomerular contractile cells.

Receptors for the majority of the vasoactive agents have been identified on the membranes of mesangial cells. These receptors are coupled to distinct signaling pathways, namely phospholipases C and A_2 and adenylyl cyclase. Vasoconstrictors like angiotensin II and arginine-vasopressin produce an increase in the intracellular calcium concentration, followed by cell contraction (9–11). Vasodilators like DA and PGE_2, which increase cAMP, induce cell relaxation (4, 12).

Recent investigations in our laboratory have demonstrated that BK binds to a specific receptor on the glomeruli (13). A

This work was supported by funds from INSERM and Hoechst Laboratory.

ABBREVIATIONS: DA, dopamine; PKC, protein kinase C; PGE_2, prostaglandin E_2; PMA, phorbol-12-myristate-13-acetate; BK, bradykinin; IBMX, isobutylmethylxanthine; PDE, phosphodiesterase; HBSS, Hanks' balanced salt solution; RIA, radioimmunoassay; DMSO, dimethylsulfoxide; K_f, ultrafiltration coefficient; (*R*)-PIA, (*R*)-N^6-(1-methyl-2-phenylethyl)adenosine; OAG, 1-oleoyl-2-acetyl-*rac*-glycerol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; MDCK, Madin-Darby canine kidney.

population of BK receptors have been identified on cultured mesangial cells (14). Activation of the BK receptor on these cells induced both stimulation of PGE_2 release (14) and inositol-1,4,5-trisphosphate production (15). However, the delicate cross-talk associating these two signaling pathways has not yet been elucidated.

The mechanism of action of BK seems to be different *in vivo*, compared with *in vitro*, because in *in vivo* studies the interaction between cells of different types cannot be ignored. *In vivo* BK acts as a vasodilator because of its action on vascular cells, especially endothelial cells, and *in vitro* experiments on cultured mesangial cells show that BK can activate both phospholipase C and phospholipase A_2 pathways, indicating that it can act either as a vasoconstrictor or as a vasodilator. Whatever the nature of these discrepancies between *in vivo* and *in vitro* studies, mesangial cells in culture resemble other vascular smooth muscle cells and represent a model for the study of the perivascular cells of the glomerulus, i.e., the cells of the afferent and efferent arterioles.

The mechanism by which BK decreases the glomerular K_f is not well understood. The present work was undertaken to examine the effect of the activation of BK receptors in isolated rat glomeruli and in cultured rat mesangial cells on basal intracellular cAMP content and intracellular cAMP content stimulated by forskolin, PGE_2, or isoproterenol. We demonstrated that an increase of cAMP level, resulting either from direct stimulation of adenylyl cyclase or via receptor-mediated stimulation, is inhibited in the presence of BK. This BK effect is induced neither by direct inhibition of adenylyl cyclase activity nor by stimulation of cAMP degradation, because it persists in the presence of IBMX, a PDE inhibitor. This BK effect is pertussis toxin independent. Furthermore, direct activation of PKC with PMA or with a diacylglycerol analog (OAG) mimicked the effect of BK on forskolin-stimulated cAMP accumulation. Also, inhibition of PKC, either directly or by down-regulation, abolished the inhibitory effect of BK on forskolin-stimulated cAMP level.

Materials and Methods

Isolation of glomeruli. Male Sprague Dawley rats weighing approximately 250 ± 20 g, fed with a normal diet (UAR A04) and water *ad libitum*, were sacrificed by decapitation. The kidneys were immediately removed and placed in ice-cold saline. The glomeruli were obtained as described previously (16). Briefly, after removal of the renal capsule the cortex was dissected away with scissors and passed successively through 180-, 125-, and 75-μm steel sieves. The glomeruli retained on the top of the 75-μm sieve were washed off with HBSS. After centrifugation (500 × *g*, 2 min at 4°) the supernatant was removed and the glomeruli were resuspended in fresh ice-cold HBSS. By this method, we obtained about 15,000 glomeruli from one kidney. Under a light microscope, >95% of the glomeruli appeared to be decapsulated and devoid of afferent and efferent arterioles.

Cell culture. Mesangial cells were prepared and cultured as described previously (14). Briefly, primary mesangial cells were obtained as outgrowths of decapsulated collagenase-digested glomeruli. Under sterile conditions the rat kidneys were removed and glomeruli were obtained as described above. Glomerular explants were allowed to grow to confluence, at 37° in a humidified atmosphere of 5% CO_2, in RPMI 1640 medium containing 15% fetal calf serum, 50 units/ml penicillin, 50 μg/ml streptomycin, 2 mM glutamine, and D-valine sustituted for L-valine to prevent fibroblast development (17). With this methodology mesangial cells appeared in the culture after about 2–3 weeks. For subculture, confluent cells were treated with trypsin-EDTA and transferred to the same complete medium. Cells between subcultures 2 and 5 were used for the experiments. Mesangial cells were identified by morphological and functional criteria as described previously (15).

Incubation protocols and assays for cAMP. For the experiments on whole glomeruli, about 2500 glomeruli/tube were incubated in HBSS with or without the different drugs, in a final volume of 400 μl. The incubations were carried out at 37° in the presence of a continuous stream of 95% O_2/5% CO_2. At the end of the incubation time the reaction was stopped by removal of the medium, followed immediately by the addition of 1.6 ml of an ice-cold 95% methanol/5% formic acid solution to each tube. For extraction of cAMP the suspensions were sonicated and centrifuged at 2500 × *g* for 15 min at 4°. The supernatants were then evaporated and cAMP was measured by RIA (ERIA diagnostic Pasteur cAMP RIA kit).

For the experiments on cultured mesangial cells, cells were seeded into six-well culture trays (Nunc) at a density of 5×10^4 cells/well and were cultured with complete medium for 48 hr. Before experiments the cells were washed three times with 1 ml/well HBSS.

Time course experiments showed that cAMP production reached a maximum at 5 min and remained stable up to 10 min. Thus, all the incubations were carried out for 5 min. When added, IBMX was preincubated for 5 min before the treatment with the different agents. Data are expressed as the amount of cAMP (measured at 5 min)/mg of protein.

The solvent for the phorbol ester and diacylglycerol analog (OAG) was DMSO, at a final concentration of 0.01%. In all experiments, simultaneous controls were performed in which glomeruli or mesangial cells were exposed only to the 0.01% DMSO solvent. This concentration of DMSO was without effect on cAMP formation.

Measurement of the cAMP-dependent PDE activity. The cAMP-dependent PDE activity was assayed on both glomeruli and mesangial cell homogenates according to the method described by De Mazancourt and Giudiceli (18), with slight modifications. Briefly, the glomeruli or mesangial cell suspensions were homogenized in a Potter apparatus in 5 ml of buffer and were then centrifuged at 20,000 × *g* for 10 min at 4°. The pellet was resuspended in 1 mM $KHCO_3$ for measurement of the cAMP-dependent PDE activity. Thirty to 50 μg of protein were incubated at 37° for 15 min in a final volume of 200 μl containing 0.5 μM cAMP, 10 nM [^{3}H]cAMP, 5 mM $MgCl_2$, 30 mM Tris·HCl, pH 7.4, and 0.04% bovine serum albumin. The reaction was stopped by heating at 95° for 3 min and the 5′-AMP was precipitated by addition of 200 μl of 0.24 M $Ba(OH)_2$ and 200 μl of 0.24 M $ZnSO_4$. After centrifugation at 5000 × *g* for 10 min, 200 μl of the supernatant were counted. A heat-denatured (3 min at 95°) homogenate was used as blank. After the blank value was subtracted, the cAMP-dependent PDE activity was expressed in pmol of cAMP transformed/mg of protein/min.

Assay of adenylyl cyclase activty. The adenylyl cyclase activity of mesangial cell plasma membrane (generally 25–50 μg of protein/assay) was measured using a modification of the method of Alvarez and Donald (19). Confluent cells were washed three times with ice-cold phosphate-buffered saline and then scraped into 2 ml of Tris buffer (50 mM Tris·HCl, pH 7.4, 5 mM EDTA). The plasma membrane suspension was obtained as described previously (13, 14), and under such experimental conditions BK binds to the plasma membrane. Plasma membrane was incubated in the presence of the following components (final concentrations): 40 mM Tris, 1.5 mM $MgCl_2$, 1 μM GTP, 100 μM EGTA, 0.5 mM IBMX, 1 mM cAMP, 5 mM creatinine phosphate, 5 mM creatinine kinase, 2 mg/ml bovine serum albumin, 0.2 mM ATP, and about 1×10^6 cpm of [α-^{32}P]ATP, in a final volume of 100 μl. After 10 min at 37°, the reaction was stopped by addition of 20 μl of [^{3}H]cAMP and heating to 100° for 4 min. [^{3}H]cAMP (about 9000 cpm) was used for recovery determination. The [^{32}P]cAMP produced was separated in a one-step elution on alumina columns.

Assay of diacylgycerol formation. Mesangial cells were plated at 50×10^3 cells/well in six-well plates. After a 48-hr period the cells were rendered quiescent by incubation for an additional 48-hr period

in the same growth medium containing only 0.5% fetal calf serum. The fetal calf serum was necessary to maintain cell viability, as demonstrated by the trypan blue exclusion test in preliminary experiments. Cells were labeled with 1 μCi/ml [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid for 24 hr in serum-free medium. They were then washed with prewarmed RPMI 1640 medium (37°) just before being stimulated with BK (10^{-7} μM) for various times. The incubations were stopped by two rapid washes with 2 ml of cold phosphate-buffered saline. The cells were dissolved by addition of 1 ml of 0.1% sodium dodecyl sulfate to each dish. Carrier lipids including monoacylglycerol and diacylglycerol (100 μg of each) were added to the combined cell extracts. Lipids were extracted with a mixture of chloroform/methanol (2:1). The organic phase was washed with chloroform/methanol/0.1 M KCl (6:92:96), dried under nitrogen, and redissolved in chloroform. Diacylglycerols were separated by thin layer chromatography using silica gel 60 plates. The solvent system used was hexane/diethyl ether/acetic acid (30:70:1). The standard used for chromatography was 1,2-[^{3}H]diacylglycerol. Radioactive diacylglycerols were visualized by iodine vapor and by autoradiography and were scraped from the plates into vials; the amount of radioactivity was quantified by liquid scintillation counting.

Protein determination. In each experiment, samples of glomerulus suspension or cell culture were used for determination of protein content. After solubilization for 15 min at 100° with 1 M NaOH, proteins were measured using the method of Lowry *et al.* (20), with bovine serum albumin as standard.

Statistical analysis. Results were expressed as mean ± standard error of at least three to five separate experiments. An unpaired *t* test or analysis of variance was performed when appropriate, and in all comparisons differences were considered significant at $p < 0.05$.

Materials. Peptides and drugs for this work came from the following sources. Collagenase was from Boehringer Mannheim, and bisindolylmaleimide was from Calbiochem. Penicillin, streptomycin, BK, IBMX, forskolin, pertussis toxin, (*R*)-PIA, PMA, 4α-phorbol-12,13-didecanoate, OAG, staurosporine, PGE_2, isoproterenol, creatinine phosphokinase, alumina, GTP, and ATP were purchased from Sigma Chemical Co. (St. Louis, MO). RPMI 1640 medium was from GIBCO-BRL. Fetal calf serum was from Sera-lab. [5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid (150–230 Ci/mmol), [α-^{32}P]ATP (10–30 Ci/mmol), and [^{3}H]cAMP (30–50 Ci/mmol) were purchased from Amersham. Plastic culture material was from Nunc and BK antagonists were a generous gift of Dr. D. Regoli (University of Sherbrooke, Quebec). HOE-140 (D-Arg^0-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK) was a generous gift of Prof. B. Schölkens (Hoechst, AG Frankfurt).

Results

Effect of BK on forskolin-stimulated cAMP production in isolated glomeruli and mesangial cells. As shown in Fig. 1a, the basal level of cAMP in the glomeruli was 4.95 ± 0.6 pmol/mg/5 min. Addition of increasing concentrations of BK from 10 nM to 10 μM did not significantly modify this basal level (data not shown). In isolated glomeruli (Fig. 1a), stimulation of cAMP production by 0.1 μM forskolin (9.45 ± 1.25 pmol/mg/5 min) was inhibited in a dose-dependent manner by BK; complete inhibition was achieved with 1 μM BK, compared with the basal level (4.93 ± 0.8 versus 4.95 ± 0.6 pmol/mg/5 min). Because the BK-induced decrease in cAMP content could result from a stimulation of cAMP degradation by PDE, the experiments were repeated in the presence of the PDE inhibitor IBMX (used at 0.5 mM), to completely inhibit PDE activity (21). In a preliminary experiment we determined the effects of IBMX from 0.1 to 1 mM on the basal level of cAMP; no significant difference was observed between 0.45 and 1 mM under our experimental conditions. As shown in Fig. 1b, IBMX increased the basal level from 4.95 ± 0.6 to 16.4 ± 1.5 pmol/mg/5 min ($p < 0.01$); forskolin raised this level to 42 ± 4 pmol/mg/5 min ($p < 0.01$) but the same inhibitory effect of BK was observed. Similar experiments were carried out on mesangial cells and, as shown in Fig. 1c, 0.1 μM BK had no inhibitory effect on the basal cAMP level (20.4 ± 3.2 pmol/mg/5 min) but inhibited the forskolin-stimulated cAMP level (21.1 ± 1.8 versus 39.2 ± 2.5 pmol/mg/5 min, $p < 0.01$). Furthermore, in the presence of IBMX (Fig. 1d) the basal level of cAMP increased to 75.6 ± 6.2 pmol/mg/5 min ($p < 0.01$) and 0.1 μM BK also induced a significant inhibition (55.5 ± 5 versus 75.6 ± 6.2 pmol/mg/5 min, $p < 0.05$). Additionally, when the cAMP level was stimulated by forskolin in the presence of IBMX, BK still produced a significant inhibition (82.3 ± 5.1 versus 105 ± 9.2 pmol/mg/5 min, $p < 0.01$). In contrast, 0.1 μM BK was without effect on the cAMP PDE activity in isolated glomeruli and mesangial cells (Table 1). Because BK was without effect on PDE activity and because the inhibitory effect of BK persisted in the presence of IBMX, the inhibitory effect of BK on the stimulated intracellular cAMP level cannot be mediated via the stimulation of cAMP degradation.

Effect of BK on adenylyl cyclase activity. To further assess whether BK produces direct inhibition of adenylyl cyclase, we studied the effect of BK on basal and stimulated adenylyl cyclase activity. As shown in Fig. 2, forskolin (0.1 μM) induced an increase in adenylyl cyclase activity, reaching 4.6 ± 0.9 pmol/mg/min, compared with the basal value of 3.1 ± 0.8 pmol/mg/min ($p < 0.05$). Preliminary experiments showed that increasing the concentration of BK was without effect on basal adenylyl cyclase activity and, interestingly, BK did not inhibit the stimulating effect of 0.1 μM forskolin on adenylyl cyclase activity (Fig. 2). Because it has been demonstrated that glomeruli (22) and mesangial cells (23) possess adenosine A1 receptors that are coupled to adenylyl cyclase through the inhibitory G protein G_i, and to provide a positive control, we have stimulated our preparations with the adenosine A1 agonist (*R*)-PIA at 1 μM. As expected, (*R*)-PIA inhibited the basal activity level by about 20% and the stimulating effect of forskolin by nearly 50%.

Effect of BK on PGE_2- and isoproterenol-stimulated cAMP production. As shown in Fig. 3, PGE_2 (10^{-10} to 10^{-6} M) induced a dose-dependent increase in cAMP content of isolated glomeruli, from 5.1 ± 0.45 to 23 ± 4.1 pmol/mg/5 min ($p < 0.01$). When BK (0.1 μM) was incubated simultaneously with increasing amounts of PGE_2, a reduction of the stimulating effect of PGE_2 on cAMP production was observed at each concentration studied ($p < 0.01$). At low concentrations of PGE_2 (10^{-9} M), BK completely inhibited the increase of cAMP production (6 ± 0.5 versus 10 ± 0.9 pmol/mg/5 min), whereas significant but incomplete inhibition by BK was observed in the presence of higher concentrations of PGE_2. With mesangial cells, as shown in Fig. 4, simultaneous incubation of BK with PGE_2 or isoproterenol also resulted in a significant 20–30% ($p < 0.05$) inhibition of the PGE_2- and isoproterenol-stimulated intracellular cAMP levels. As already mentioned for Fig. 2, to provide a positive control we stimulated the mesangial cells with 1 μM (*R*)-PIA. The A1 receptor agonist inhibited basal and hormone-stimulated cAMP production (Fig. 4).

Inhibition of the effect of BK by specific BK antagonists. The effects of BK on cAMP levels of isolated glomeruli and cultured mesangial cells in the presence of various antagonists are summarized in Table 2. All of the specific B2 antagonists tested prevented BK inhibition of cAMP production.

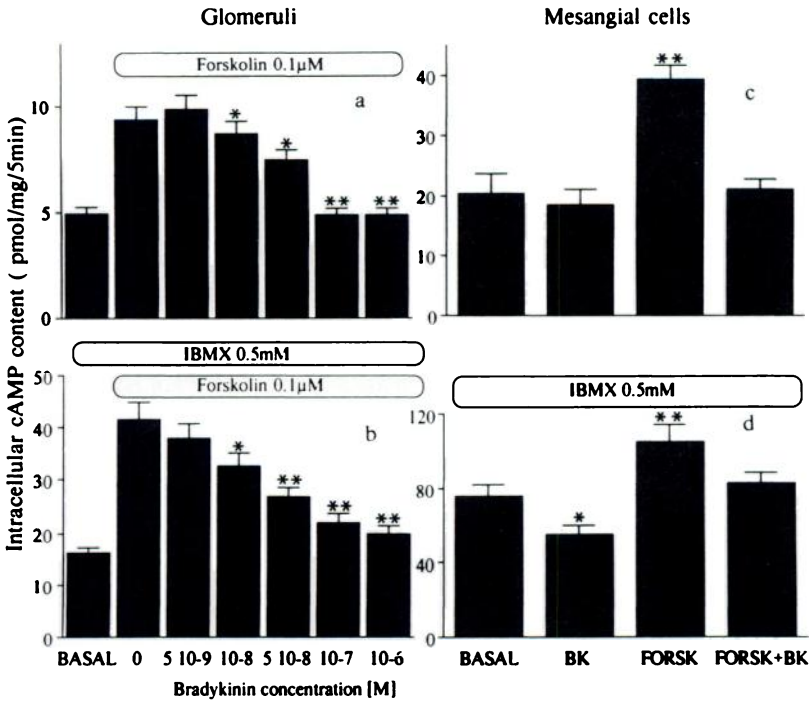

Fig. 1. Effect of BK on forskolin-stimulated cAMP content of whole glomeruli and cultured mesangial cells. Isolated rat glomeruli and cultured mesangial cells were obtained as described in Materials and Methods. Glomeruli or mesangial cells were incubated for 5 min at 37°. a, Glomeruli were stimulated with 10^{-7} M forskolin in the presence of the indicated concentrations of BK. b, Same experiment as described in a was performed in the presence of 0.5 mM IBMX, a PDE inhibitor. c, Mesangial cells were stimulated with 10^{-7} M BK, 10^{-7} M forskolin (*FORSK*), or the mixture of forskolin plus BK. d, Experiments identical to those in c were performed but 0.5 mM IBMX, a PDE inhibitor, was added to the incubation medium. Intracellular cAMP was measured by RIA, as described in Materials and Methods. Results are means ± standard errors. Each *point* represents the mean of 16 individual values from four experiments. In each experiment, each *point* was determined in quadruplicate. *, $p < 0.05$; **, $p < 0.01$, compared with forskolin alone (a and b) or basal (c and d).

TABLE 1

Effect of BK on cAMP-dependent PDE activity in isolated glomeruli and in cultured mesangial cells

Isolated rat glomeruli and cultured mesangial cells were obtained and the cAMP PDE activity was determined as described in Materials and Methods. The results are means ± standard errors. Each point represents the mean of nine individual values from three experiments.

	cAMP PDE activity	
	Control	BK (10^{-7} M)
	pmol/min/mg of protein	
Glomeruli	166 ± 27	143 ± 28
Mesangial cells	110 ± 13	95 ± 10

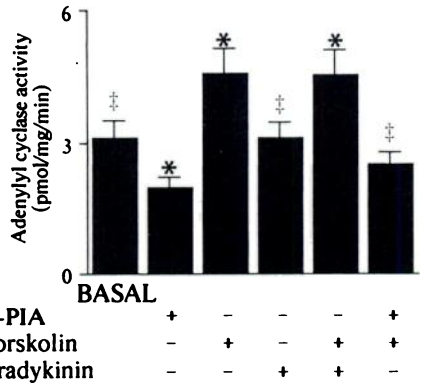

Fig. 2. Effect of (*R*)-PIA (A1 agonist), BK, and forskolin on adenylyl cyclase activity of mesangial cells. Cultured mesangial cells were obtained and adenylyl cyclase activity was measured as described in Materials and Methods. Plasma membranes of mesangial cells were incubated in the presence of 1 μM (*R*)-PIA, 10^{-7} M forskolin, or 10^{-7} M BK, alone or together, as indicated. Results are means ± standard errors. Each *point* represents the mean of 12 individual values from three experiments. In each experiment, each *point* was obtained in quadruplicate. *, $p < 0.05$, compared with the basal value; ‡, $p < 0.05$, compared with forskolin alone.

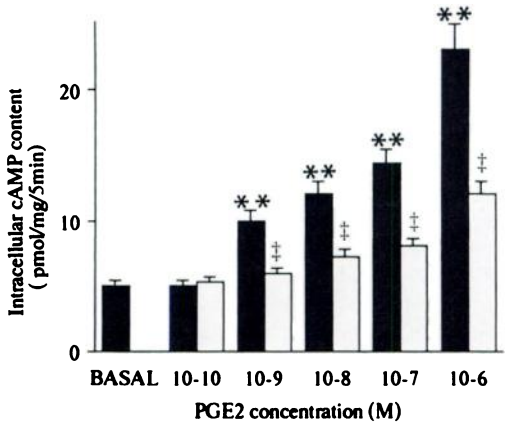

Fig. 3. Effect of BK on PGE_2-induced stimulation of cAMP content in whole glomeruli. Isolated rat glomeruli were obtained by graded sieving as described in Materials and Methods. Glomeruli were incubated for 5 min at 37°. They were stimulated by increasing concentrations of PGE_2 alone (■) or in the presence of 10^{-7} M BK (□). Intracellular cAMP was measured by RIA as described in Materials and Methods. Results are means ± standard errors. Each *point* represents the mean of 12 individual values from three experiments. In each experiment each *point* was determined in quadruplicate. **, $p < 0.01$, compared with the basal value; ‡, $p < 0.01$, compared with PGE_2 alone at each concentration.

Because [des-Arg^9,Leu^8]-BK, a specific B1 receptor antagonist, has no effect and because BK can demonstrate partial B1 agonist properties, we can reasonably postulate that only a B2 kinin receptor is involved in the BK inhibition of cAMP production.

Action of pertussis toxin on the BK inhibitory effect. Pretreatment of the mesangial cells with pertussis toxin (0.1 μg/ml for 18 hr) as described previously (24, 25), which could increase cAMP via inhibition of G_i, resulted, as expected (Fig. 5a), in an enhanced basal level (33.8 ± 3.3 versus 20.4 ± 3.2 pmol/mg/5 min, $p < 0.05$). However, BK inhibited this pertussis toxin-induced increase (25.1 ± 2.2 versus 33.8 ± 3.3 pmol/mg/5 min). Moreover, in cells pretreated with pertussis toxin,

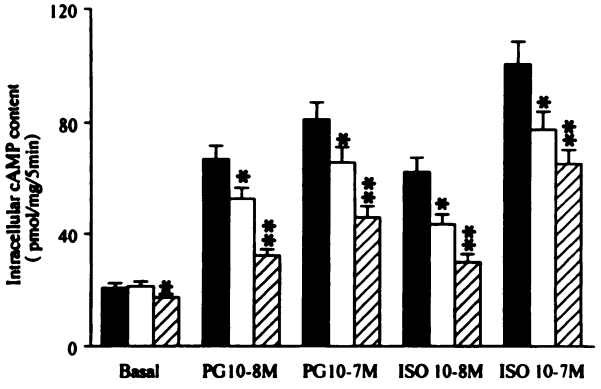

Fig. 4. Effect of (*R*)-PIA (A1 agonist) and BK on PGE_2 (*PG*)- and isoproterenol (*ISO*)-induced stimulation of cAMP content in cultured mesangial cells. Mesangial cells were obtained as described in Materials and Methods. They were stimulated with PGE_2 or isoproterenol at two different concentrations (10^{-8} M and 10^{-7} M), alone (■) or in the presence of 10^{-7} M BK (□) or 1 μM (*R*)-PIA (▨). Intracellular cAMP was measured by RIA as described in Materials and Methods. Results are means ± standard errors. Each *point* represents the mean of 12 individual values from three experiments. In each experiment each *point* was obtained in quadruplicate. *, $p < 0.05$; **, $p < 0.01$, compared with the respective control (■) in the absence of BK or (*R*)-PIA.

BK still reduced the PGE_2- or isoproterenol-dependent increases in cAMP (Fig. 5b). The pretreatment with pertussis toxin completely abolished the effect of (*R*)-PIA on basal or hormone-stimulated cAMP production (data not shown).

Effect of PMA on BK-induced inhibition of basal cAMP in the presence of IBMX. To explore the mechanism by which BK inhibits basal cAMP accumulation in the presence of IBMX, we studied the effect of short term treatment with 100 nM PMA, a concentration known to activate PKC. PMA alone produced an inhibition of cAMP accumulation (62 ± 5.5 versus 75.6 ± 6.2 pmol/mg/5 min) similar to that of BK alone (Fig. 6). Simultaneous addition of PMA and BK did not produce an additive effect. Moreover, the biologically inactive phorbol ester 4α-phorbol-12,13-didecanoate was without effect.

Effect of mepacrine, a prostaglandin synthesis inhibitor. We previously demonstrated that BK stimulated PGE_2 release from mesangial cells (14) and, because PGE_2 can act through a receptor associated with the inhibition of adenylyl cyclase (26), this endogenous, newly synthesized PGE_2 could mediate the inhibitory effect of BK on cAMP accumulation. To check this hypothesis, we incubated mesangial cells with the phospholipase A_2 inhibitor mepacrine. Mepacrine alone was without effect on the basal level of cAMP and did not change the inhibitory effect of BK on cAMP levels, indicating that under our experimental conditions endogenous PGE_2 was not involved (data not shown).

Effect of PKC activity modulators on BK-mediated inhibition of cAMP accumulation. On whole glomeruli (Table 3), direct stimulation of PKC with either PMA or the diacylglycerol analog OAG (2.10^{-5} M) resulted in an inhibition of forskolin-stimulated cAMP formation (4.3 ± 0.2 for OAG and 5.5 ± 0.9 for PMA versus 9.5 ± 1.3 pmol/mg/5 min for forskolin alone) similar to that produced by BK. However, the inhibitory effects of BK and OAG or PMA were not additive. Moreover, 4α-phorbol-12,13-didecanoate was without effect on forskolin-stimulated cAMP and did not alter the inhibitory effect of BK. On the other hand, inhibition of PKC activity with staurosporine (10^{-8} M) or bisindolylmaleimide (10^{-8} M) completely abolished the inhibitory effect of BK on the forskolin-induced increase of cAMP content. When the experiments were repeated with the mesangial cells (Fig. 7), stimulation of PKC with either OAG or PMA or inhibition of its activity with staurosporine or bisindolylmaleimide resulted in the same effect as those described with whole glomeruli. Moreover, when mesangial cells were treated with a phorbol ester (160 nM PMA) for 18 hr to produce down-regulation of PKC (27), the basal level of cAMP was increased (37.3 ± 5 versus 20.4 ± 3.2 pmol/mg/5 min) and the inhibitory effect of BK was no longer observed (40.6 ± 4 versus 37.3 ± 5 pmol/mg/5 min). Moreover, in such PKC-down-regulated cells 10^{-7} M forskolin induced an increase in cAMP levels that was not inhibited by 10^{-7} M BK.

Effect of BK on diacylglycerol production. Because we previously showed (15) that BK stimulates inositol trisphosphate formation in mesangial cells and because our present data show that OAG mimics the effect of BK, we investigated the effect of BK (10^{-7} M) on diacylglycerol production (Table 4). As expected, after 1 min of incubation BK stimulated diacylglycerol production, reaching a maximum level after 5 min. As a consequence, it can be seen that the inhibitory effect of BK on the forskolin-stimulated cAMP level follows the time course of diacylglycerol production.

Discussion

The present study demonstrates that, in both isolated glomeruli and mesangial cells, BK receptor activation inhibits the

TABLE 2

Effect of specific BK antagonists on the inhibitory effect of BK on forskolin-stimulated cAMP content

Isolated rat glomeruli and mesangial cells were obtained as described in Materials and Methods. Glomeruli or mesangial cells were incubated for 5 min at 37°. The different antagonists are all B2 antagonists except for [des-Arg⁹-Leu⁸]-BK, which is a specific B1 antagonist. All antagonists were tested at 10^{-5} M. Forskolin and BK used were at 10^{-7} M. Intracellular cAMP was measured by RIA as described in Materials and Methods. Results are means ± standard errors. Each point represents the mean of nine individual values from three experiments. In each experiment, each point was obtained in triplicate.

Additions	cAMP			
	Glomeruli		Mesangial cells	
	Forskolin	Forskolin + BK	Forskolin	Forskolin + BK
	pmol/mg/5 min			
Control	9.45 ± 1.25	5.2 ± 0.7[a]	39.2 ± 2.5	21.1 ± 1.8[a]
[D-Arg,Hyp³,Thi⁵,⁸,D-Phe⁷]-BK	9.2 ± 1.4	11.4 ± 0.8	40 ± 3	39.8 ± 1.7
[D-Arg,Hyp³,D-Phe⁷]-BK	10.1 ± 1.1	9.8 ± 1.3	39.3 ± 2.7	35.3 ± 4
[Thi⁵,⁸,D-Phe⁷]-BK	9.6 ± 2	8.7 ± 0.65	38 ± 1.8	36 ± 2.6
HOE-140	9.1 ± 0.5	10.3 ± 0.9	41 ± 3.2	38.7 ± 2
[Des-Arg⁹,Leu⁸]-BK	9.8 ± 0.3	5.5 ± 1[a]	39.5 ± 2.2	22.4 ± 1.37[a]

[a] $p < 0.05$, compared with forskolin alone.

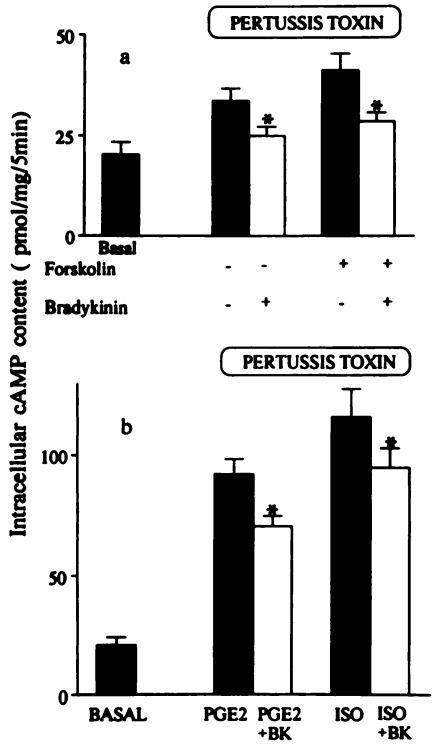

Fig. 5. Effect of pretreatment with pertussis toxin on the BK inhibitory effect. Mesangial cells were obtained as described in Materials and Methods. a, Mesangial cells were pretreated with pertussis toxin (100 ng/ml) for 18 hr and then stimulated with either 10^{-7} M forskolin or 10^{-7} M BK or both. b, Mesangial cells were pretreated with pertussis toxin (100 ng/ml) for 18 hr and then incubated with either PGE_2 or isoproterenol (*ISO*) at 10^{-7} M, alone (■) or with BK (10^{-7} M) (□). Intracellular cAMP was measured by RIA as described in Materials and Methods. Results are means ± standard errors. Each *point* represents the mean of nine individual values from three experiments. In each experiment, each *point* was obtained in triplicate. *, $p < 0.05$, compared with the respective control (■).

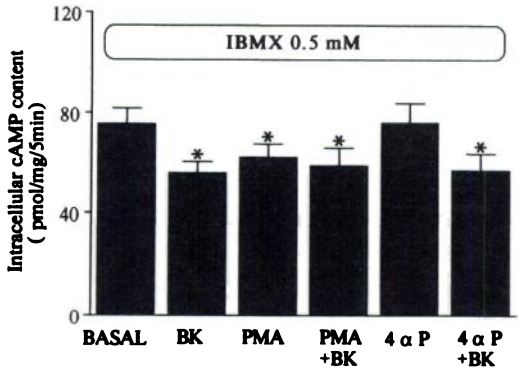

Fig. 6. Effect of PMA and 4α-phorbol-12,13-didecanoate on BK-induced inhibition of basal cAMP content in cultured mesangial cells. Mesangial cells were obtained as described in Materials and Methods. They were pretreated for 5 min with PMA (100 nM) or 4α-phorbol-12,13-didecanoate (100 nM), in incubation medium containing IBMX (0.5 mM), before stimulation for 5 min at 37° with BK (10^{-7} M), PMA, or 4α-phorbol-12,13-didecanoate (*4 α P*) alone or a mixture of PMA plus BK or 4α-phorbol-12,13-didecanoate plus BK. Results are means ± standard errors. Each *point* represents the mean of nine individual values from three experiments. In each experiment each *point* was obtained in triplicate. *, $p < 0.05$, compared with the basal value.

TABLE 3

Effect of PKC activators and inhibitors on the inhibitory effect of BK on forskolin-stimulated cAMP content in whole glomeruli

Isolated rat glomeruli were obtained as described in Materials and Methods. They were incubated for 5 min at 37°. OAG (diacylglycerol analog) was used at 2×10^{-5} M, staurosporine and bisindolylmaleimide at 10^{-8} M, PMA at 100 nM, and 4α-phorbol-12,13-didecanoate at 100 nM. BK and forskolin were used at 10^{-7} M. Forskolin (10^{-7} M) gave 190% of the basal value. Intracellular cAMP was measured by RIA as described in Materials and Methods. Results are means ± standard errors. Each point represents the mean of nine individual values from three experiments. In each experiment each point was obtained in triplicate.

	cAMP	Inhibition of forskolin (10^{-7} M)-stimulated level
	pmol/mg/ 5 min	%
Basal	4.95 ± 0.6[a]	0
Staurosporine	5.3 ± 0.5[a]	0
Bisindolylmaleimide	5.5 ± 0.4[a]	0
Forskolin (10^{-7} M)	9.45 ± 1.25[b]	0
Forskolin + BK	5.2 ± 0.7[a]	45 ± 8[a]
Forskolin + OAG	4.3 ± 0.2[a]	54.5 ± 6[a]
Forskolin + OAG + BK	4.5 ± 0.35[a]	52.4 ± 12[a]
Forskolin + PMA	5.5 ± 0.9[a]	42 ± 7[a]
Forskolin + PMA + BK	5.1 ± 0.5[a]	46 ± 6[a]
Forskolin + 4α-phorbol-12,13-didecanoate	9.8 ± 1.8[b]	0.03 ± 0.01
Forskolin + 4α-phorbol-12,13-didecanoate + BK	4.9 ± 0.8[a]	48 ± 8[a]
Forskolin + staurosporine + BK	10.2 ± 2.2[b]	0.08 ± 0.01
Forskolin + bisindolylmaleimide + BK	10.7 ± 1.5[b]	0.13 ± 0.01

[a] $p < 0.05$, compared with forskolin alone.
[b] $p < 0.05$, compared with basal values.

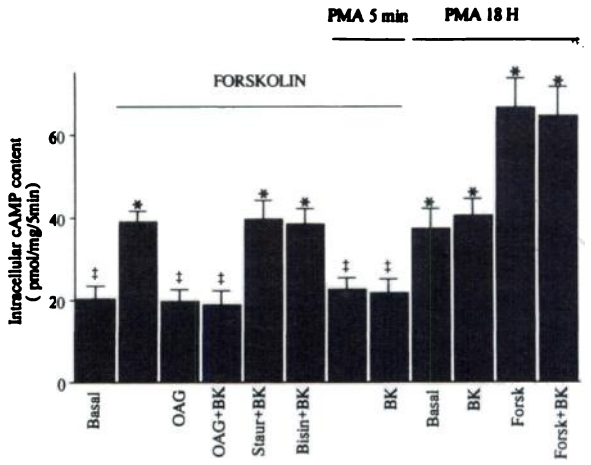

Fig. 7. Effect of modulation of PKC activity on the BK-induced inhibition of forskolin-stimulated cAMP content in mesangial cells. Mesangial cells were obtained as described in Materials and Methods. Direct stimulation of PKC was obtained by pretreating the cells with PMA (100 nM) for 5 min at 37° before the addition of forskolin (*Forsk*) (10^{-7} M) or forskolin plus BK (10^{-7} M) for 5 min at 37°. Down-regulation of PKC was obtained by pretreatment of the cells with PMA (160 nM) for 18 hr. Down-regulation of PKC could not be achieved on isolated glomeruli, which cannot be maintained under isolated conditions for 18 hr. Stimulations were performed with different mixtures of agents as indicated (OAG, 2×10^{-5} M; *Staur*, staurosporine, 10^{-8} M; *Bisin*, bisindolylmaleimide, 10^{-8} M). Intracellular cAMP was measured by RIA as described in Materials and Methods. Results are means ± standard errors. Each *point* represents the mean of nine individual values from three experiments. In each experiment each *point* was obtained in triplicate. *, $p < 0.01$, compared with the basal value; ‡, $p < 0.01$, compared with forskolin.

TABLE 4

Time course of effect of BK on formation of diacylglycerol and cAMP production

Mesangial cells were obtained as described in Materials and Methods. They were labeled with [^{3}H]arachidonic acid for 24 hr and then stimulated with BK (10^{-7} M) for various times. The time course of the accumulation of cAMP was determined with 10^{-7} M forskolin alone or with a mixture of 10^{-7} M forskolin plus 10^{-7} M BK, for various times. Samples were then analyzed as described in Materials and Methods. Results are means ± standard errors. Each point represents the mean of nine individual values from three experiments.

Time	BK	Diacylglycerol	cAMP	
			Forskolin	Forskolin + BK
min	*dpm*	*% of basal*	*pmol/mg*	
0	1230 ± 100		9.7 ± 0.7	9.5 ± 0.6
0.5	1414 ± 150	115 ± 11	15 ± 1.3	13 ± 1.1
1	1635 ± 145[a]	133 ± 9	25 ± 1.9	18 ± 1.4[b]
5	1722 ± 220[a]	140 ± 13	39 ± 3.5	20 ± 1.5[b]
10	1780 ± 210[a]	145 ± 12	38 ± 3.2	19.8 ± 2[b]

[a] $p < 0.05$, compared with 0 min.
[b] $p < 0.05$, compared with the respective forskolin time alone.

accumulation of intracellular cAMP induced by direct activation of adenylyl cyclase by either forskolin, PGE_2, or isoproterenol, all known to increase intracellular cAMP levels via activation of specific membrane receptors (12, 28). Because the same inhibitory effect of BK on cAMP accumulation was observed on isolated glomeruli and on mesangial cells, this effect cannot be linked to cell culture conditions. This effect of BK is mediated by the activation of a specific B2 kinin receptor, because the decrease in cAMP accumulation is blocked by specific B2 receptor antagonists but not by the specific B1 receptor antagonist [des-Arg^9,Leu^8]-BK. The inhibitory effect of BK on cAMP accumulation is largely mediated through activation of PKC and is independent of activation of phospholipase A2 and of the involvement of pertussis toxin-sensitive G_i. However, these findings raise several points that deserve discussion, particularly with respect to the sites and mechanisms of inhibition.

An inhibitory effect of BK on cAMP accumulation has previously been described in cultured collecting tubular cells after isoproterenol or vasopressin (24) stimulation, as well as through activation of PKC on MDCK cells (29). This inhibitory effect of BK on cAMP production has also been demonstrated in neuronal NCB20 (25) and N1E-115 cells (30). In contrast, stimulation of cAMP formation through a PGE_2-dependent mechanism has been reported in arterial smooth cells (31). However, the effect of BK on cAMP had never been investigated in mesangial cells; cAMP is considered to be an important modulator of the contractile state of these cells, which may play a role in the regulation of glomerular filtration. Taken together, the previous studies and the present data indicate that, depending upon the cell type, BK can either stimulate or inhibit cAMP formation, suggesting several sites of action.

A decrease in cAMP level can result either from the stimulation of cAMP degradation by PDE or from the inhibition, directly or via the coupling system, of its formation by adenylyl cyclase. In the present study, the direct stimulation of PDE activity, resulting in an increased degradation of cAMP, is unlikely because BK is without effect on PDE activity and the inhibitory effect of BK persists in the presence of the PDE inhibitor IBMX. Therefore, it appears likely that BK inhibits cAMP formation.

BK is known to increase PGE_2 production in mesangial cells (14, 32), and in turn PGE_2 can stimulate but also inhibit adenylyl cyclase through a G_i-coupled receptor (26). Although a stimulating effect of exogenous PGE_2 on cAMP accumulation was observed in the present study, the possibility of an inhibitory action of endogenous PGE_2 cannot be eliminated. The participation of endogenously formed PGE_2 in the inhibitory effect of BK on cAMP formation is unlikely, because blockade of phospholipase A_2 activity with the selective inhibitor mepacrine did not prevent BK inhibition of forskolin-stimulated cAMP production. Furthermore, these results are consistent with the study of Dominguez *et al.* (33), which demonstrated that PGE_2 inhibits adenylyl cyclase in the proximal tubule but not in the glomeruli. Our results are also in agreement with other reports that have shown that treatment with a phospholipase A_2 inhibitor does not prevent the inhibitory effect of BK on vasopressin-stimulated cAMP accumulation in cortical collecting tubular cells and MDCK cells (24, 29).

Forskolin stimulation is inhibited by BK, suggesting that inhibition could occur directly on adenylyl cyclase. However, BK had no inhibitory effect on the *in vitro* activity of adenylyl cyclase before or after forskolin stimulation. This absence of a direct effect of BK on adenylyl cyclase activity has already been reported in other cell types, i.e., arterial smooth muscle cells (31) and NCB-20 cells (25). The possibility of a direct inhibitory effect on the adenylyl cyclase subunit cannot be dismissed, but the effect may require cytoplasmic constituents.

Another possiblity for decreasing the intracellular cAMP level could be stimulation of G_i. To test this hypothesis, G_i was inhibited with pertussis toxin for 18 hr. In pertussis toxin-treated cells, BK still inhibited the cAMP accumulation induced by either forskolin, PGE_2, or isoproterenol, indicating that the inhibitory effect of BK is pertussis toxin independent. These results are in agreement with previous studies reporting pertussis toxin-insensitive BK effects (15, 24, 30, 34, 35). However, the involvement of a pertussis toxin-insensitive G protein cannot be rejected.

Now it is seems clear that inhibition of cAMP accumulation is mediated through activation of PKC. The involvement of PKC is demonstrated by (i) the direct stimulation of PKC by PMA inducing the same inhibitory effect on forskolin-stimulated cAMP as BK, (ii) the lack of effect of the inactive phorbol ester 4α-phorbol-12,13-didecanoate, and (iii) the suppression of the inhibitory effect of BK by two selective PKC inhibitors (staurosporine and bisindolylmaleimide). On the other hand, we showed that BK stimulated diacylglycerol production. Moreover, the diacylglycerol analog OAG, an activator of PKC, inhibited forskolin-stimulated cAMP production on both glomeruli and cultured mesangial cells with the same magnitude as BK. However, the inhibitory effects of OAG and BK on cAMP accumulation were not additive. The involvement of PKC is confirmed in mesangial cells pretreated with the phorbol ester PMA. In the presence of IBMX, short term treatment with PMA produced an effect on basal cAMP similar to that of BK, and 4α-phorbol-12,13-didecanoate was without effect. A long pretreatment (18 hr), known to completely desensitize PKC (27), produced an increase in the basal level of cAMP, suggesting that, under normal conditions, PKC could act on processes that lead to a decrease of intracellular cAMP content. Further studies will be required to determine the precise mechanism of this effect. In rabbit collecting tubular cells (24) and in MDCK cells (29), a similar inhibitory effect of BK on cAMP formation is blocked by PKC inhibitors acting on the hormone

receptor or on the coupling of the receptor to G_s. However, none of these previous studies demonstrated an inhibition of forskolin-stimulated cAMP synthesis, which suggests differences in the sites of inhibition for BK depending on the cell model. Our results are in agreement with those of Dixon *et al* (36), who reported that OAG produces rapid inhibition of vasopressin- and forskolin-stimulated adenylyl cyclase activity. Taken together, these results suggest that the BK effect is mediated by the stimulation of phospholipase C, leading to the generation of diacylglycerol and subsequently the activation of PKC, which then inhibits the catalytic subunit of adenylyl cyclase.

Several studies have indicated that activation of PKC leads to the attenuation of ligand-stimulated cAMP production in a variety of cell systems (for review, see Ref. 37). The inhibition can result from phosphorylation of the binding site or uncoupling from the stimulatory G protein (38). This mechanism has been suggested to explain the inhibitory effect of BK on hormone-stimulated cAMP production in other cell types (24, 29). In our study, the inhibitory effect of BK on PGE_2 and isoproterenol-stimulated cAMP accumulation probably operates by this mechanism and not by the activation of a pertussis toxin-insensitive G protein as reported for N1E-115 cells (30), where the toxin-insensitive response was not mediated by PKC, unlike in our results.

In our study, the effect of PMA is consistent with that obtained with OAG. However, other studies (29, 36, 39) have reported that only OAG, and not PMA, prevented forskolin-stimulated cAMP accumulation. The discrepancies between these data and our study could be explained by the use of different cellular models. Furthermore, it has been shown (40) that different activators of PKC stimulate different physiological responses in GH_4 pituitary cells, suggesting that, although OAG and phorbol ester are effective activators of PKC, other effects induced by these two agents could be involved in the modulation of cAMP production.

Finally, this inhibitory effect of BK on cAMP accumulation brings complementary information to our understanding of mesangial BK receptor activation, which is associated with inositol-1,4,5-trisphosphate formation (15), an increase in intracellular calcium (41), and also stimulated PGE_2 secretion (14). Therefore, we report for the first time that BK, via a B2 kinin receptor, inhibits forskolin-, PGE_2-, and isoproterenol-stimulated cAMP accumulation in isolated glomeruli and cultured mesangial cells. This effect is mediated neither by direct inhibition of adenylyl cyclase activity nor by stimulation of cAMP degradation by PDE. It is mimicked by PMA and blocked by two selective PKC antagonists. Moreover, BK stimulates diacylglycerol production and OAG produces inhibition of forskolin-stimulated cAMP, whereas the down-regulation of PKC by long term pretreatment with PMA abolishes the inhibitory effect of BK on forskolin-stimulated cAMP levels. Neither prostaglandins nor the inhibitory G protein appear to mediate this effect. Taken together, these results suggest that activation of the BK receptor on glomeruli or mesangial cells leads to the activation of phospholipase C and subsequently to the activation of PKC, which then inhibits hormone- or forskolin-stimulated cAMP accumulation. Further studies are necessary to elucidate the mechanism by which PKC inhibits basal and forskolin-stimulated cAMP production in whole glomeruli and in mesangial cells. The existence of isoforms of both adenylyl cyclase and PKC raises the possibility of different interactions between them.

Acknowledgments

We thank Dr. P. Winterton for revising the English version.

References

1. Latta, H. Ultrastructure of the glomerular and juxtaglomerular apparatus, *Handbook of Physiology*. Sec. 7. Chap. 1. American Physiological Society, Washington, DC, 1–29 (1973).
2. Kreisberg, J. I., M. A. Venkatachalam, and D. Troyer. Contractile properties of cultured mesangial cells. *Am. J. Physiol.* **249**:F457-F463 (1985).
3. Anchews, P. M., and A. K. Coeffey. Cytoplasmic contractile elements in glomerulus cells. *Fed. Proc.* **42**:3046–3052 (1983).
4. Mene, P., F. Pugliese, and G. A. Cinotti. Cellular basis of hormonal actions in the glomerular. *Am. J. Nephrol.* **10** (Suppl. 1):28–35 (1990).
5. Baylis, C., K. M. Deen., B. D. Myers, and B. M. Brenner. Effects of some vasodilator drugs on transcapillary fluid exchange in renal cortex. *Am. J. Physiol.* **230**:1148–1158 (1976).
6. Baylis, C., H. G. Rennke, and B. M. Brenner. Mechanism of the defect in glomerular ultrafiltration associated with gentamicin administration. *Kidney Int.* **12**:344–353 (1977).
7. Dworking, L. D., I. Ichikawa, and B. M. Brenner. Hormonal modulation of glomerular function. *Am. J. Physiol.* **244**:F95-F104 (1983).
8. Sharshmidt, L. A., J. G. Douglas, and M. J. Dunn. Angiotensin II and eicosanoids in the control of glomerular size in the rat and human. *Am. J. Physiol.* **250**:F348-F356 (1986).
9. Ausiello, D. A., J. I. Kreisberg, C. Royand, and M. J. Karnovsky. Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. *J. Clin. Invest.* **65**:754–760 (1980).
10. Bonventre, J. V., K. Skorecki, J. I. Kreisberg, and J. Y. Cheung. Vasopressin increases cytosolic free calcium concentration in glomerular mesangial cells. *Am. J. Physiol.* **251**:F94-F102 (1986).
11. Hassid, A., N. Pidikiti, and D. Gamero. Effects of vasoactive peptides on cytosolic calcium in cultured mesangial cells. *Am. J. Physiol.* **251**:F1018-F1028 (1986).
12. Mene, P., and M. J. Dunn. Eicosanoids and control of mesangial cell contraction. *Circ. Res.* **62**:916–925 (1988).
13. Bascands, J. L., C. Pecher, G. Cabos, and J. P. Girolami. B2-kinin receptor like binding in rat glomerular membranes. *Biochem. Biophys. Res. Commun.* **158**:99–104 (1989).
14. Emond, C., J. L. Bascands, C. Pecher, G. Cabos-Boutot, P. Pradelles, D. Regoli, and J. P. Girolami. Characterization of a B2-kinin receptor in rat renal mesangial cells. *Eur. J. Pharmacol.* **190**:381–392 (1990).
15. Bascands, J. L., C. Emond, C. Pecher, D. Regoli, and J. P. Girolami. Bradykinin stimulates production of inositol (1,4,5) trisphosphate in cultured rat mesangial cells via B2-kinin receptor. *Br. J. Pharmacol.* **102**:962–966 (1991).
16. Emond, C., J. L. Bascands, G. Cabos-Boutot, C. Pecher, and J. P. Girolami. Effect of changes in sodium or water intake on glomerular B2-kinin binding sites. *Am. J. Physiol.* **257**:F353-F358 (1989).
17. Gilbert, S. F., and B. R. Migeon. D-Valine as a selective agent for normal human and epithelial cells in culture. *Cell* **5**:11–17 (1975).
18. De Mazancourt, P., and Y. Giudiceli. Guanine nucleotides and adenosine 'R_i'-site analogues stimulate the membrane-bound low-K_m cyclic AMP phosphodiesterase of rat adipocytes. *FEBS Lett.* **173**:385–388 (1984).
19. Alvarez, R., and V. D. Donald. A single method for the assay of adenylate cyclase. *Anal. Biochem.* **187**:98–103 (1990).
20. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
21. Knecht, M., and K. J. Catt. Gonodotropin-releasing hormone: regulation of adenosine 3′,5′-monophosphate in ovarian granulosa cells. *Science (Washington D. C.)* **214**:1346–1348 (1981).
22. Liu, Y., S. Umemura, N. Hirawa, Y. Toya, and M. Ishii. Biphasic effects of an adenosine analogue on the cyclic AMP formation in isolated rat glomeruli. *Genet. Hypertens.* **218**:487–489 (1992).
23. Olivera, A., M. Tomas, and J. M. Lopez-Nova. Effect of adenosine A1 and A2 agonists and antagonists on cAMP and Ca^{2+} in cultured rat mesangial cells. *Am. J. Physiol.* **262**:C840-C844 (1992).
24. Dixon, B. S., R. Breckon, J. Fortune, E. Sutherland, F. R. Simon, and R. J. Anderson. Bradykinin activates protein kinase C in cultured cortical collecting tubular cells. *Am. J. Physiol.* **257**:F808-F817 (1989).
25. Boyajian, C. L., A. Garritsen, and D. M. F. Cooper. Bradykinin stimulates Ca^{2+} mobilization in NCB-20 cells leading to direct inhibition of adenylate cyclase. *J. Biol. Chem.* **266**:4995–5003 (1991).
26. Sonnenberg, W. K., and W. L. Smith. Regulation of cyclic AMP metabolism in rabbit collecting tubule cells by prostaglandins. *J. Biol. Chem.* **263**:6155–6160 (1988).
27. Rodriguez-Pena, A., and E. Rozengurt. Disappearence of Ca^{2+} sensitive, phospholipid-dependent protein-kinase activity in phorbol ester-treated 3T3 cells. *Biochem. Biophys. Res. Commun.* **120**:1053–1059 (1984).

28. Troyer, D. A., O. F. Gonzalez, M. A. Venkatachalam, and J. I. Kreisberg. Elevation of cAMP in cultured mesangial cells diminishes vasopressin-stimulated increases of phosphate uptake and ^{32}P specific activity in ATP but has no effect on phosphoinositide metabolism. *J. Biol. Chem.* **262:**1614–1617 (1987).
29. Friedlander, G., and C. Amiel. Protein kinase C activators and bradykinin selectively inhibit vasopressin-stimulated cAMP synthesis in MDCK cells. *Biochim. Biophys. Acta* **929:**311–317 (1987).
30. Bozou, J. C., F. de Nadai, J. P. Vincent, and P. Kitabgi. Neurotensin, bradykinin and somatostatin inhibit cAMP production in neuroblastoma N1E-115 cells via both pertussis toxin sensitive and insensitive mechanisms. *Biochem. Biophys. Res. Commun.* **161:**1144–1150 (1989).
31. Dixon, B. S., R. Breckon, J. Fortune, R. J. Vavrek, J. M. Stewart, R. Marzec-Calvert, and S. L. Linas. Effects of kinin on cultured arterial smooth muscle. *Am. J. Physiol.* **258:**C299-C308 (1990).
32. Uglesity, A., J. I. Kreisberg, and L. Levine. Stimulation of arachidonic acid metabolism in rat kidney mesangial cells by bradykinin, antidiuretic hormone and their analogues. *Prostaglandins Leukotrienes Med.* **10:**83–93 (1983).
33. Dominguez, J. H., F. Shuler, M. W. Olszowy, T. Brown, and J. B. Pushett. Prostaglandin E_2 is an inhibitor of adenylate cyclase in rabbit proximal tubule. *Am. J. Physiol.* **254:**C304-C309 (1988).
34. Kremer, S., P. Harper, R. Hegele, and K. Skorecki. Bradykinin stimulates a rise in cytosolic calcium in renal glomerular mesangial cells via a pertussis toxin insensitive pathway. *Can. J. Physiol. Pharmacol.* **66:**43–48 (1987).
35. Jackson, T. R., S. I. Patterson, Y. H. Wong, and M. R. Handley. Bradykinin stimulation of inositol phosphate and calcium responses is insensitive to pertussis toxin in NG115–401L neuronal cells. *Biochem. Biophys. Res. Commun.* **148:**412–416 (1987).
36. Dixon, B. S., R. Breckon, C. Burke, and R. J. Anderson. Phorbol esters inhibit adenylate cyclase activity in cultured collecting tubular cells. *Am. J. Physiol.* **254:**C183-C191 (1988).
37. Houslay, M. D. "Crosstalk": a pivotal role for protein kinase C in modulating relationships between signal transduction pathways. *Eur. J. Biochem.* **195:**9–27 (1991).
38. Nambi, P., J. R. Peters, D. R. Sibley, and R. J. Lefkovitz. Desensitization of the turkey erythrocyte β-adrenergic receptor in a cell-free system: evidence that multiple protein kinases can phosphorylate and desensitize the receptor. *J. Biol. Chem.* **260:**2163–2171 (1985).
39. Shinohara, O., M. Knecht, and K. J. Catt. Differential actions of phorbol ester and diacylglycerol on inhibition of granulosa cell maturation. *Biochem. Biophys. Res. Commun.* **133:**468–474 (1985).
40. Ramsdell, J. S., G. R. Petit, and A. H. Tashjian. Three activators of protein kinase C, bryostatins, dioleins and phorbol esters, show differing specificities of action on GH_4 pituitary cells. *J. Biol. Chem.* **261:**17073–17080 (1986).
41. Bascands, J. L., C. Pecher, S. Rouaud, C. Emond, J. Leung-Tack, M. J. Bastie, R. Burch, D. Regoli, and J. P. Girolami. Evidence for the existence of two distinct BK-receptors on rat mesangial cells. *Am. J. Physiol.* **264:**F548-F556 (1993).

Send reprint requests to: J. P. Girolami, INSERM (CJF 92.05), Institut Louis Bugnard, School of Medicine Rangueil, Toulouse 31062 Cedex, France.