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Different effects of phorbol ester on angiotensin II- and stable GTP analogue-induced activation of polyphosphoinositide phosphodiesterase in membranes isolated from rat renal mesangial cells

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Pretreatment with pertussis toxin inhibits angiotensin II-induced activation of polyphosphoinositide phosphodiesterase in rat renal mesangial cells [Pfeilschifter & Bauer (1986) Biochem. J. **236**, 289–294]. Furthermore, activation of protein kinase C by the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and by 1-oleoyl-2-acetylglycerol (OAG) abolishes angiotensin II-induced formation of inositol trisphosphate (IP_3) in mesangial cells [Pfeilschifter (1986) FEBS Lett. **203**, 262–266]. Using membrane preparations of [^{3}H]inositol-labelled mesangial cells we tried to obtain further insight as to the step at which protein kinase C might interfere with the signal transduction mechanism in mesangial cells. Angiotensin II (100 nM) stimulates IP_3 formation from membrane preparations of [^{3}H]inositol-labelled mesangial cells with a half-maximal potency of 1.1 nM. The angiotensin II-induced formation of IP_3 is enhanced by GTP. This effect of angiotensin II is completely blocked by the competitive antagonist [Sar^1,Ala^8]angiotensin II. Guanosine 5'-[γ-thio]triphosphate (GTPγS) and guanosine 5'-[$\beta\gamma$-imido]triphosphate (Gpp[NH]p), non-hydrolysable analogues of GTP, stimulate IP_3 production in the absence of angiotensin II with K_d values of 0.19 μM and 2.4 μM, respectively. Angiotensin II augments the increase in IP_3 formation induced by GTPγS. However, when mesangial cells were pretreated with TPA there was a dose-dependent inhibition of the synergistic action of angiotensin II on GTPγS-induced IP_3 production. Comparable results are obtained with OAG, while the non-tumour-promoting phorbol ester 4α-phorbol 12,13-didecanoate is without effect. These results suggest that activation of protein kinase C in mesangial cells does not impair phosphoinositide hydrolysis by stable GTP analogues but somehow seems to interfere with the stimulatory interaction of the occupied angiotensin II receptor with the transducing G-protein.

INTRODUCTION

The action of various agonists that mediate physiological responses through calcium mobilization coincides with their ability to activate a phosphodiesterase that hydrolyses PIP_2 to generate the two second messengers IP_3 and DG (for review see [1–3]). Recent evidence has suggested that guanine nucleotide-binding regulatory proteins may be important in calcium-mediated cell responses. In 1983 Gomperts showed that introduction of Gpp[NH]p into permeabilized mast cells stimulated histamine release in the presence of calcium [4]. Stable analogues of GTP also enhanced calcium-stimulated serotonin release and DG formation in permeabilized platelets [5,6]. Recently evidence was presented for a guanine nucleotide effect on PIP_2 phosphodiesterase activity in different cell systems [7–13]. Comparable results were obtained by us using rat mesangial cells [14–16]. We have shown that vasoconstrictors, such as angiotensin II, vasopressin and noradrenaline, caused a rapid breakdown of PIP_2 in rat mesangial cells with concomitant production of DG and IP_3 [14,15]. Furthermore we have shown that pretreatment of mesangial cells with pertussis toxin abolished the angiotensin II-induced breakdown of PIP_2 and it was suggested that a guanine nucleotide-binding regulatory protein is involved in receptor-mediated activation of polyphosphoinositide phosphodiesterase in mesangial cells [16].

Recent studies have demonstrated that several agonist-induced processes in different cell types can be inhibited by preincubation with tumour-promoting phorbol esters, suggesting a role for protein kinase C as a negative feedback regulator of cell function [17–23]. The results obtained with mesangial cells fit well in this scheme [24]. There have been striking similarities in the action of TPA and pertussis toxin in mesangial cells: both inhibited dose- and time-dependently phosphoinositide hydrolysis and $[Ca^{2+}]_i$ rises without affecting angiotensin II binding to intact cells [16,24]. Therefore we speculated that TPA and pertussis toxin may have the same target, i.e. a G-protein coupling angiotensin II receptors to polyphosphoinositide phosphodiesterase.

In this paper we try to obtain further insight into the mode of action of protein kinase C as a negative

Abbreviations used: IP_3, inositol trisphosphate; IP_2, inositol bisphosphate; IP_1, inositol monophosphate; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; OAG, 1-oleoyl-2-acetylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate; DG, 1,2-diacylglycerol; GTPγS, guanosine 5'-[γ-thio]-triphosphate; Gpp[NH]p, guanosine 5'-[$\beta\gamma$-imido]triphosphate; GDPβS, guanosine 5'-[β-thio]diphosphate; $[Ca^{2+}]_i$, intracellular free calcium concentration; MEM, minimal essential medium; PBS, phosphate-buffered saline.

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feedback regulator in mesangial cells. We present evidence from studies using *myo*-[^{3}H]inositol-labelled membranes that angiotensin II and GTP act synergistically to stimulate phosphodiesterase-mediated hydrolysis of PIP_2. Our data further indicate that pretreatment of mesangial cells with TPA does not impair PIP_2 hydrolysis by stable GTP analogues but strongly inhibits the synergistic action of angiotensin II on IP_3 production.

EXPERIMENTAL PROCEDURES

Materials

Angiotensin II, [Sar^1,Ala^8]angiotensin II, TPA and 4α-phorbol 12,13-didecanoate were purchased from Sigma. OAG was purchased from Molecular Probes Inc. (Eugene, OR, U.S.A.). *myo*-[2-^{3}H]Inositol was purchased from Amersham International and guanine nucleotides were purchased from Boehringer Mannheim. All other chemicals not specifically mentioned were from Merck.

Cell culture

Rat mesangial cells were cultivated as described previously [25]. The first passage of mesangial cells was used for all experiments. The cells were grown in RPMI 1640 (Boehringer) supplemented with 10% (v/v) fetal bovine serum (Boehringer), penicillin (100 units/ml), streptomycin (100 μg/ml) (Boehringer) and bovine insulin (0.66 units/ml) (Sigma). Tissue flasks and dishes (Greiner, Nürtingen, Germany) were incubated in a humidified atmosphere in incubators in air/CO_2 (19:1).

Cell labelling and preparation of cell membranes

Cells were prelabelled for 72 h with *myo*-[2-^{3}H]inositol (20 μCi/ml) in MEM free of inositol, containing 10% dialysed fetal bovine serum. After this prelabelling period the medium was removed and the cells were rinsed several times with PBS and labelled cells were removed from bottles by incubation in PBS without Mg^{2+} or Ca^{2+}, but containing 2 mM-EDTA and trypsin (0.25%, w/v). Suspended cells were subsequently washed twice with cold MEM by low-speed centrifugation and resuspended in cold 'lysis buffer' (320 mM-sucrose, 0.5 mM-EDTA, 25 mM-LiCl, 100 μM-ATP and 10 mM-Tris/HCl, pH 7.8 at 0 °C). In experiments where the effect of phorbol esters was investigated, the cells were incubated with the indicated concentrations of TPA, 4α-phorbol 12,13-didecanoate, OAG or vehicle for 10 min at 37 °C prior to the addition of lysis buffer. The cells were homogenized by 40 strokes of a Dounce homogenizer. Membranes were isolated by centrifugation for 5 min at 15000 *g* in an Eppendorf microfuge, resuspended in lysis buffer at a protein concentration of about 4 mg/ml and used immediately.

Incubation of membranes and whole cells and analysis of inositol phosphates

For whole cell experiments, cells were grown on culture dishes (7 cm^2) and labelled for 72 h, then were rinsed several times to remove free [^{3}H]inositol. Cells were then incubated for various times in 1 ml of MEM with or without angiotensin II. After the different times the medium was aspirated and the reactions were terminated by the addition of 1 ml of 15% (w/v) trichloroacetic acid. The trichloroacetic acid was removed by extraction with diethyl ether. The final extract was neutralized and applied to anion-exchange columns containing 1 ml of Dowex 1X8 (100–200 mesh, formate form; Serva, Heidelberg, Germany). Free inositol and inositol phosphates were eluted sequentially according to the method of Berridge [26] as described [15]. Incubation of membranes was started by the addition of 25 μl of membrane suspension to 125 μl of incubation buffer. The final composition of incubation buffer was: 53 mM-sucrose, 0.08 mM-EDTA, 1.7 mM-Tris/HCl, 1.2 mM-$MgCl_2$, 1 μM free Ca^{2+}, 29 mM-LiCl and 50 mM-sodium phosphate buffer (pH 7.0) plus addition of angiotensin II or other agents. Incubation was carried out at 37 °C for the indicated time and was terminated by the addition of 1.25 ml of methanol/chloroform (2:1, v/v). A further 0.5 ml of chloroform and 0.5 ml of water were added and the lipids were extracted for 1 h at room temperature. After centrifugation the upper phase was removed and the lower phase was washed twice with 200 μl of fresh upper phase as described by Litosch *et al.* [8]. The combined aqueous phases were centrifuged for 30 min in a Speedvac to remove remaining methanol and separated by anion exchange chromatography as described above.

Additional methods

Protein was determined by the method of Lowry [27] with bovine serum albumin (Sigma) as standard.

RESULTS

Angiotensin II stimulates inositol phosphates production in mesangial cells

Angiotensin II causes a rapid increase in the formation of IP_3, IP_2 and IP_1 in intact mesangial cells (Fig. 1). IP_3 levels were highest at 10 s after exposure of the cells to angiotensin II (the earliest time point measured), whereas

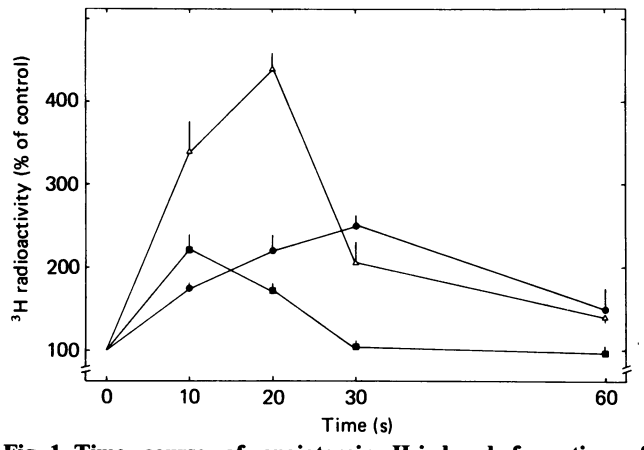

Fig. 1. Time course of angiotensin II-induced formation of inositol phosphates in intact mesangial cells

Mesangial cells were labelled with [^{3}H]inositol as described under 'Experimental procedures'. At each time point IP_3 (■), IP_2 (△) and IP_1 (●) levels were determined in the absence or in the presence of 100 nM-angiotensin II. Data are expressed as percentage of control ± S.E.M. for four experiments. The average c.p.m./mg of cell protein in the absence and presence of angiotensin II at 10 s were, respectively: 181 ± 17 and 398 ± 34 for IP_3, 438 ± 39 and 1489 ± 164 for IP_2, 1270 ± 87 and 2210 ± 95 for IP_1.

IP_2 levels peak at 20 s and those of IP_1 at 30 s. This is in good agreement with the earlier reported kinetics of angiotensin II-induced PIP_2 hydrolysis and DG formation [15]. Half-maximal stimulation of IP_3 formation occurs at approx. 3.5 nM-angiotensin II (Fig. 2). Maximal stimulation of IP_3 formation is achieved at 100 nM-angiotensin II.

Angiotensin II-induced hydrolysis of membrane phosphoinositides requires guanine nucleotides

Using incubations of membranes prepared from [^{3}H]-inositol-labelled mesangial cells with no experimental additions, there is an observed generation of IP_3 and IP_2. These studies are done in the presence of LiCl which inhibits phosphatase-mediated hydrolysis of IP_1 to free inositol. Fig. 3 illustrates the time course of the effects of GTP (100 μM), angiotensin II (100 nM) and GTP plus angiotensin II on the accumulation of [^{3}H]IP_3 and [^{3}H]IP_2. Addition of angiotensin II stimulates IP_3 and IP_2 formation, whereas addition of GTP alone has no significant influence. This might indicate that the reaction system is still charged with enough endogenous GTP to allow angiotensin II to act up to a certain degree.

In contrast, when angiotensin II and GTP are added together IP_3 and IP_2 generation is stimulated to a greater extent than with angiotensin II alone (Fig. 3). There is only a little IP_1 formed (50–100 c.p.m. in the different preparations) and this amount of IP_1 does not increase with time (results not shown). This probably reflects the fact that phosphatidylinositol does not serve as a substrate for the membrane-bound polyphosphoinositide phosphodiesterase. Furthermore an IP_2 phosphatase seems not to be present in this membrane preparation, as no further breakdown of IP_2 could be detected. Half-maximal stimulation of the production of inositol phosphates occurs at 1.1 nM-angiotensin II in the presence of 100 μM-GTP (Fig. 4). This is well within the concentration range where angiotensin II causes half-maximal stimulation of inositol phosphates in intact cells (Fig. 2). Maximal effects on IP_3 formation are seen at 100 nM-angiotensin II. The effect of angiotensin II is completely blocked by the competitive antagonist [Sar^1,Ala^8]angiotensin II. As shown in Table 1, in the presence of saturating concentrations of [Sar^1,Ala^8]-angiotensin II, angiotensin II is without effect on production of inositol phosphates. Hydrolysis-resistant GTP analogues stimulate IP_3 production in the absence of angiotensin II (Fig. 5). In particular, GTPγS is highly active, as previously reported for other membrane systems [4–8,10,12,13]. Gpp[NH]p is also effective, although to a lesser extent (Fig. 6). GTPγS causes a 210% increase in IP_3 generation; half-maximal stimulation is found in the presence of 190 nM-GTPγS. Gpp[NH]p causes an 88% increase in IP_3 formation, half-maximal stimulation occurring in the presence of 2.4 μM-Gpp[NH]p. Control experiments indicated that GDP and GMP have no influence on inositol phosphate generation (results not shown). The stable GDP analogue

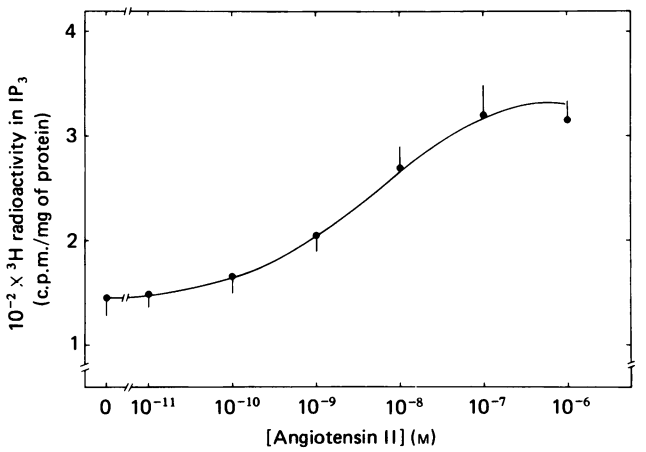

Fig. 2. Dose-dependency of angiotensin II-induced formation of IP_3 in intact mesangial cells

Mesangial cells were labelled with [^{3}H]inositol as described under 'Experimental procedures'. Mesangial cells were exposed to the indicated concentrations of angiotensin II for 10 s. IP_3 was determined as described under 'Experimental procedures'. Results are expressed as ^{3}H radioactivity/mg of cell protein and are the means ± S.E.M. for four experiments.

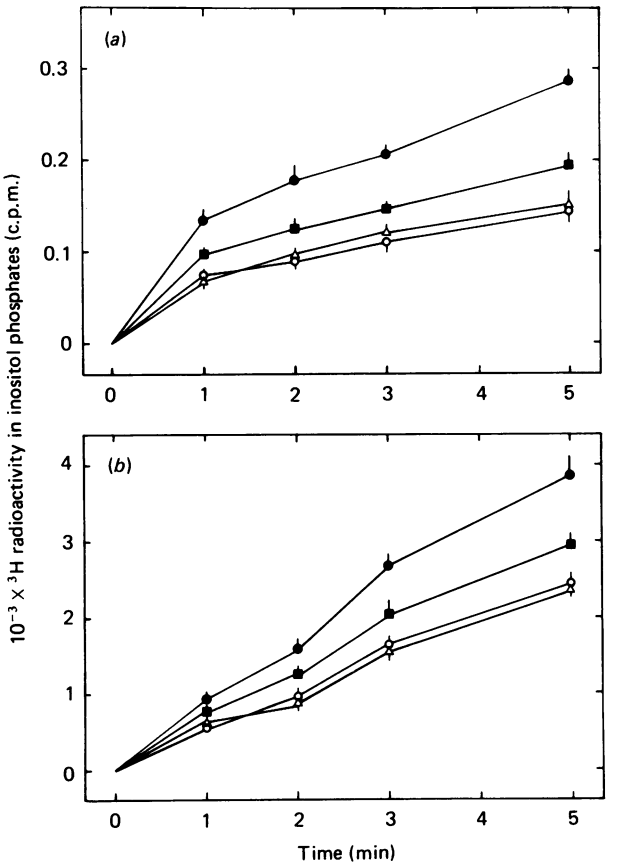

Fig. 3. Time course of the effects of angiotensin II, GTP and angiotensin II plus GTP on [^{3}H]IP_3 (*a*) and [^{3}H]IP_2 (*b*) formation in membrane suspensions of mesangial cells

Membranes prepared from mesangial cells prelabelled with [^{3}H]inositol were incubated at 37 °C in buffer containing 100 μM-ATP without (control, ○) or with 100 nM-angiotensin II (■), 100 μM-GTP (△) or 100 nM-angiotensin II plus 100 μM-GTP (●). The incubation was terminated at the indicated times by the addition of methanol/chloroform and IP_3 and IP_2 were separated as described under 'Experimental procedures'. The results are means ± S.E.M. for four experiments.

GDPβS effectively antagonizes stimulation of IP_3 formation by GTPγS, as shown in Table 2. Stimulation by 100 μM-GTPγS is completely suppressed by GDPβS when the latter nucleotide is present in a 10-fold molar excess.

Phorbol ester treatment impairs angiotensin II-induced activation of PIP_2 phosphodiesterase

Like GTP, the stable analogue GTPγS augments the increase in IP_3 production due to angiotensin II. As shown in Fig. 7(*a*), half-maximal stimulation of angiotensin II (100 nM)-induced IP_3 formation occurs at approx. 400 nM-GTPγS. However, when mesangial cells are pretreated with TPA (100 nM) for 10 min, a treatment which has been shown to suppress maximally angiotensin II-induced effects in whole cells [24], there is a strong inhibition of the synergistic action of angiotensin II on guanine nucleotide-induced IP_3 formation (Fig. 7*b*). This effect of TPA is concentration-dependent, as shown in Fig. 8. The concentration of TPA needed (1–100 nM) is in the range believed to cause specifically an activation of protein kinase C [3]. Furthermore, the biologically

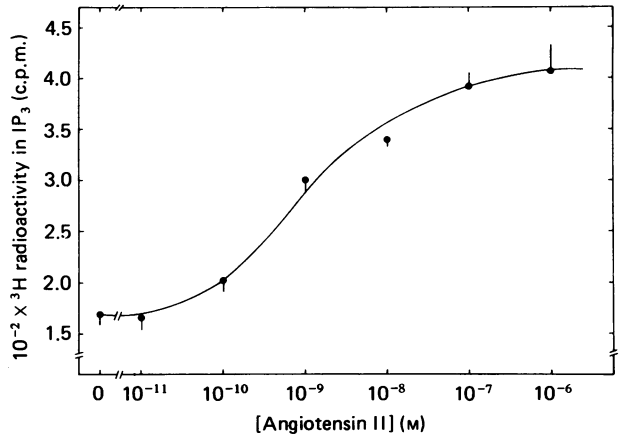

Fig. 4. Dose-dependency of angiotensin II-induced formation of [^{3}H]IP_3 in membrane suspension of mesangial cells

Membranes prepared from mesangial cells prelabelled with [^{3}H]inositol were incubated at 37 °C in buffer containing 100 μM-ATP, 100 μM-GTP and the indicated concentration of angiotensin II. The incubations were terminated after 5 min by adding methanol/chloroform and IP_3 was separated as described under 'Experimental procedures'. The results are mean ± S.E.M. for four experiments.

Table 1. Inhibition of the angiotensin II-stimulated IP_3 formation in membranes of mesangial cells by the competitive antagonist [Sar^1,Ala^8]angiotensin II

Membranes prepared from mesangial cells prelabelled with [^{3}H]inositol were incubated at 37 °C for 5 min in buffer containing 100 μM-ATP, 100 μM-GTP with or without 10 nM-angiotensin II or 100 nM-[Sar^1,Ala^8]-angiotensin II. The incubations were terminated by adding methanol/chloroform and IP_3 was separated as described under 'Experimental procedures'. The results are means ± S.E.M. for four experiments.

Addition	IP_3 formation (c.p.m.)
None	187 ± 18
Angiotensin II (10 nM)	341 ± 14
[Sar^1,Ala^8]Angiotensin II (100 nM)	197 ± 22
Angiotensin II (10 nM) + [Sar^1,Ala^8]angiotensin II (100 nM)	202 ± 25

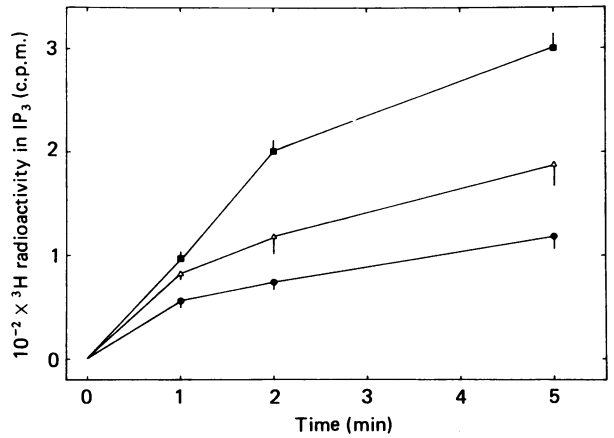

Fig. 5. Time course of [^{3}H]IP_3 formation in response to non-hydrolysable GTP analogues in membrane suspensions of mesangial cells

Membranes were prepared from [^{3}H]inositol-prelabelled mesangial cells and incubated for the indicated time in the absence of guanine nucleotides (control, ●) or in the presence of either 100 μM-Gpp[NH]p (△) or 10 μM-GTPγS (■). Reactions were terminated and IP_3 levels determined as described under 'Experimental procedures'. Data are expressed as c.p.m. of ^{3}H and are means ± S.E.M. for four experiments.

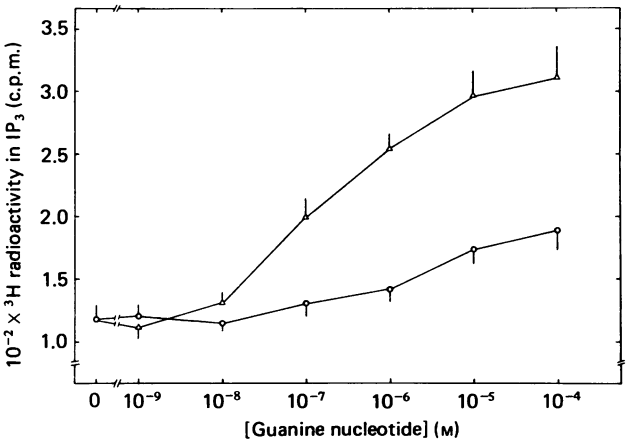

Fig. 6. Gpp[NH]p and GTPγS concentration-dependent stimulation of [^{3}H]IP_3 formation in membrane suspension of mesangial cells

Membranes were prepared from [^{3}H]inositol-prelabelled mesangial cells as described under 'Experimental procedures'. IP_3 levels were determined after incubation at 37 °C for 5 min in the presence of the indicated concentrations of either Gpp[NH]p (○) or GTPγS (△). Results are means ± S.E.M. for four experiments.

Table 2. Antagonism of GTPγS-induced IP_3 formation by GDPβS

Membranes were prepared from [^{3}H]inositol-prelabelled mesangial cells as described under 'Experimental procedures'. Membranes were incubated at 37 °C for 5 min with 100 μM-GTPγS with or without 1 mM-GDPβS. Reactions were stopped by the addition of methanol/chloroform and IP_3 was separated as described under 'Experimental procedures'. The results are means ± S.E.M. for four experiments.

Additions	IP_3 formation (c.p.m.)	
	No GDPβS	GDPβS (1 mM)
None	118 ± 12	125 ± 15
GTPγS (100 μM)	311 ± 25	132 ± 18

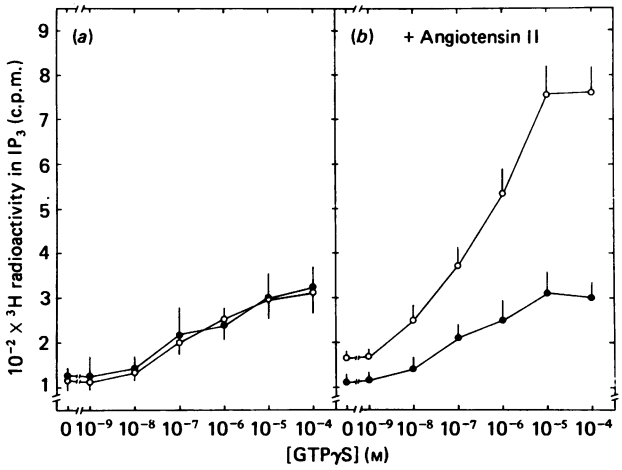

Fig. 7. Dose-dependency of GTPγS- and angiotensin II-induced formation of [^{3}H]IP_3 from membranes of control and TPA-pretreated mesangial cells

Membranes were prepared from [^{3}H]inositol-prelabelled mesangial cells which had been pretreated with 100 nM-TPA or vehicle for 10 min as described under 'Experimental procedures'. IP_3 levels were determined after incubation for 5 min at 37 °C in the presence of the indicated concentrations of GTPγS (*a*), or with angiotensin II (100 nM) and the indicated concentrations of GTPγS (*b*). Results are means ± S.E.M. for four experiments. ○, Cells not pretreated with TPA; ●, cells pretreated with TPA.

inactive phorbol ester 4α-phorbol 12,13-didecanoate is without effect (Fig. 8), and the synthetic diacyglycerol OAG also partly inhibits this synergistic action of angiotensin II on GTPγS-evoked IP_3 formation (Table 3).

DISCUSSION

The present work indicates that in a cell-free system obtained from [^{3}H]inositol-labelled mesangial cells, guanine nucleotides and angiotensin II stimulate the formation of inositol phosphates. Thus this study directly supports our previous proposal of a role for a guanine nucleotide-binding regulatory protein in the coupling of angiotensin II receptors to polyphosphoinositide phosphodiesterase in mesangial cells [16]. As we have shown earlier, pretreatment of mesangial cells with TPA blocked the formation of IP_3 in response to angiotensin II [24]. Neither the number of angiotensin II receptors nor their affinity was affected by TPA treatment [24]. These observations suggest that the inhibitory action of TPA occurs at a step distal to receptor activation. In order to characterize further the possible target substrate of protein kinase C in mesangial cells we have pretreated mesangial cells with TPA and OAG, in concentrations believed to activate protein kinase C specifically, and have then examined the effect of GTP analogues and angiotensin II on IP_3 production in membranes prepared from these cells. We find that

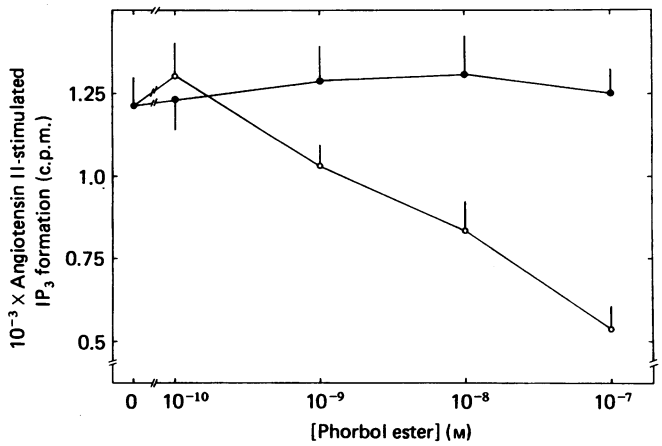

Fig. 8. Dose-dependency of inhibition of angiotensin II-induced IP_3 formation by TPA and 4α-phorbol 12,13-didecanoate

Membranes were prepared from [^{3}H]inositol-prelabelled mesangial cells which had been pretreated with the indicated concentrations of TPA (○) or 4α-phorbol 12,13-didecanoate (●) or vehicle for 10 min as described under 'Experimental procedures'. IP_3 levels were determined after incubation for 5 min at 37 °C in the presence of 100 nM-angiotensin II and 100 μM-GTPγS. Results are means ± S.E.M. for four experiments.

Table 3. Inhibition of angiotensin II-induced IP_3 formation by OAG

Membranes prepared from [^{3}H]inositol-prelabelled mesangial cells which had been pretreated with 25 μM-OAG or vehicle for 10 min as described under 'Experimental procedures' were incubated at 37 °C. IP_3 levels were determined after 5 min of incubation in the presence of 100 μM-GTPγS either with or without angiotensin II (100 nM). Results are the means ± S.E.M. for four experiments.

Additions	IP_3 formation (c.p.m.)	
	Untreated	OAG-pretreated
None	118 ± 12	109 ± 15
GTPγS (100 μM)	311 ± 25	298 ± 24
GTPγS (100 μM) + angiotensin II (100 nM)	762 ± 54	452 ± 68

pretreatment of mesangial cells does not impair the IP_3 formation induced by GTPγS, but strongly inhibits the synergistic action of angiotensin II on this GTPγS-evoked IP_3 production. Therefore it seems that protein kinase C somehow impairs the interaction of the occupied receptor with the G-protein, and therefore inhibits the agonist-induced formation of IP_3 in mesangial cells.

Possible explanations for the observed reduction in the stimulated hydrolysis of polyphosphoinositides in cells pretreated with TPA could be: (1) that protein kinase C might inhibit the dissociation of the α-subunit from the βγ-subunit by receptor stimulation; (2) that the oligomer can be dissociated by receptor stimulation but the GTP–GDP exchange at the α-subunit may not occur; and (3) that the interaction of the α-subunit with phospholipase C or phospholipase C activity itself is inhibited.

The latter two possibilities do not occur in mesangial cells, as TPA pretreatment of the cells does not inhibit GTPγS-induced IP_3 formation (Fig. 7). This also excludes a possible effect of TPA pretreatment on phospholipase C activity itself. Therefore we assume that protein kinase C inhibits the dissociation of the α-subunit from the βγ-subunit by angiotensin II stimulation in mesangial cells. Matsumoto *et al.* [28] showed in a recent paper that pretreatment of rabbit neutrophils with TPA stimulated the pertussis toxin-dependent ADP-ribosylation of a 41 kDa protein. Since only the oligomeric (αβγ) form of a G-protein can function as a substrate for pertussis toxin, the authors speculated that protein kinase C shifts the association/dissociation state of the GTP-binding protein to its oligomeric (αβγ) form. Also, Halenda *et al.* [29] demonstrated that pretreatment of human platelets with TPA somehow attenuated the maximal extent of the dissociation of G-protein oligomers normally caused by thrombin. In contrast with our observations, Orellana *et al.* [30] report that GTPγS-dependent IP_3 formation in membranes of astrocytoma cells is inhibited by TPA and protein kinase C. They conclude that the inhibitory action of TPA is exerted on a G-protein and affects its ability to interact with phospholipase C.

Astrocytoma cells have been reported to possess a pertussis toxin-insensitive G-protein that couples muscarinic receptors to phosphoinositide hydrolysis [31]. This indicates that the coupling G-protein in astrocytoma cells might be different from the G-protein coupling angiotensin II receptors to phospholipase C in mesangial cells, as the latter is sensitive to pertussis toxin [16]. The different sensitivity to pertussis toxin suggests a degree of heterogeneity in this coupling factor, and immunological estimates of the total G-protein content of cells shows that there may still be many unidentified G-proteins [32]. This might perhaps account for the different responses to phorbol ester in astrocytoma cells and in mesangial cells.

Concerning the sensitivity to pertussis toxin, the coupling G-protein in mesangial cells resembles the G_i protein of the adenylate cyclase system. In a recent review Taylor & Merritt [33] stressed the striking parallels of receptor coupling to polyphosphoinositide phosphodiesterase in comparison with the receptor-mediated regulation of adenylate cyclase. In a recent paper Bauer & Jakobs [34] showed that TPA pretreatment impairs hormone-induced, but not stable GTP analogue-induced, inhibition of adenylate cyclase. Their results are rather similar to the results we obtained for angiotensin II-induced stimulation of polyphosphoinositide phosphodiesterase in mesangial cells. Furthermore, Yamashita *et al.* [35] reported that treatment of rat reticulocytes with TPA reduced the stimulation of adenylate cyclase activity by β-adrenergic agonists, whereas little effect was seen on guanine nucleotide-stimulated adenylate cyclase.

In summary, the results presented in this paper indicate that the negative feedback regulator role of protein kinase C on the signal transduction mechanism of angiotensin II in mesangial cells is possible at the level of a guanine nucleotide-binding regulatory protein. Protein kinase C might inhibit the dissociation of the α-subunit from the βγ-subunit by receptor stimulation. Future work has to be directed towards determining the nature of the G-protein that couples the angiotensin II receptor to polyphosphoinositide phosphodiesterase in mesangial cells.

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