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Role of phospholipase C and protein kinase C in vasoconstrictor-induced prostaglandin synthesis in cultured rat renal mesangial cells

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1. It was the aim of the present study to find out if a common mechanism exists by which the vasoconstrictive hormones angiotension II, noradrenaline and 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (AGEPC) increase prostaglandin E_2 (PGE_2) synthesis in cultures of rat renal mesangial cells. 2. Angiotension II, noradrenaline and AGEPC stimulated PGE_2 synthesis and uptake of $^{45}Ca^{2+}$ in cultured mesangial cells. Both of these effects could be completely suppressed by the calcium channel blocker verapamil. 3. Angiotensin II, noradrenaline and AGEPC caused a rapid breakdown of phosphatidylinositol 4,5-bisphosphate with a concomitant increase of 1,2-diacylglycerol and inositol trisphosphate, indicating an activation of phospholipase C by these hormones. Addition of verapamil had no effect on the hormone-induced stimulation of phospholipase C. 4. The synthetic analogue of diacylglycerol, 1-oleoyl-2-acetylglycerol, and the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA), both of which are known to stimulate protein kinase C, enhanced PGE_2 synthesis. Chelation of extracellular calcium with EDTA or addition of verapamil abolished the effect of 1-oleoyl-2-acetylglycerol and phorbol ester on PGE_2 synthesis. 5. 1-Oleoyl-2-acetylglycerol and phorbol ester increased the uptake of $^{45}Ca^{2+}$ by the cells in a dose-dependent manner and this effect could be blocked by verapamil. 6. The entirety of these data leads us to suggest that vasoconstrictor-evoked synthesis of PGE_2 in rat mesangial cells is mediated by the subsequent activation of phospholipase C and protein kinase C. The activation of protein kinase C by diacylglycerol is likely to be involved in the increase of the calcium permeability of the plasma membrane which is a prerequisite for PGE_2 synthesis induced by vasoconstrictive hormones.

INTRODUCTION

Angiotensin II and AVP are thought to affect the filtration function of renal glomeruli by causing a contraction of glomerular mesangial cells (Mahieu *et al.*, 1980; Ausiello *et al.*, 1980; Scharschmidt & Dunn, 1983; Fujiwara *et al.*, 1984). Furthermore, angiotension II and AVP enhance the synthesis of vasodilatatory prostaglandins by mesangial cells which supposedly modulate the contractile effect of angiotension II and AVP (Kreisberg *et al.*, 1982; Scharschmidt & Dunn, 1983). Scharschmidt & Dunn (1983) have shown that the stimulation of PG synthesis by angiotension II and AVP in cultured renal mesangial cells strongly depends on the availability of extracellular calcium, because calcium channel blockers or the omission of extracellular calcium abolished the peptide-stimulated PG production.

It is generally agreed upon that hormones that mediate physiological responses through calcium mobilization also stimulate the metabolism of membrane phosphoinositides, i.e. the PI cycle (cf. Michell *et al.*, 1981; Michell, 1982). Recently we have shown that one of the early events in the stimulation of PG synthesis by AVP in cultured renal mesangial cells is an activation of phospholipase C as evaluated by the rapid breakdown of PIP_2 and the appearance of DG and phosphatidic acid (Pfeilschifter *et al.*, 1984). However, it is not clear in what way the requirement for extracellular calcium in prostaglandin production and the activation of phospholipase C in the vasoconstrictor-induced synthesis of PG are inter-related. The present study was carried out in order to gain some insights into the nature of the link between an activation of phospholipase C by vasoconstrictive hormones in cultured rat renal mesangial cells and the recruitment of extracellular calcium. Experimental evidence indicates that an activation of protein kinase C by DG enhances the calcium permeability of the plasma membrane which is conditional for the vasoconstrictor-induced stimulation of PGE_2 synthesis.

Part of this material was presented in abstract form at the 61st meeting of the German Physiological Society (Pfeilschifter *et al.*, 1985).

EXPERIMENTAL PROCEDURES

Cell culture

The preparation of isolated glomeruli from rat kidneys and subculture of glomerular outgrowths was done as described (Kurtz *et al.*, 1982, 1983). In brief, glomeruli from male Sprague–Dawley rats (70–100 g body wt.) were prepared under sterile conditions. The kidneys were first flushed free of blood. Renal cortical tissue was

Abbreviations used: AVP, [arginine]vasopressin; PG, prostaglandin; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-monophosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; DG, 1,2-diacylglycerol; AGEPC, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine ('platelet-activating factor'); TPA, 12-*O*-tetradecanoylphorbol 13-acetate; OAG, 1-oleoyl-2-acetylglycerol; IP_1, inositol monophosphate; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; HBS, Hepes-buffered saline; MEM, minimum essential medium.

removed and consecutively passed through polyamide screens (Verseidag, Kempen, Germany) with pore sizes of 100 μm and 180 μm onto a screen of 50 μm pore size. Preparations of glomeruli from the 50 μm screen were seeded in 75 cm^2 tissue flasks (Greiner, Nürtingen, Germany). Glomerular cell outgrowths subcultured 3 weeks after the first inoculation were used for all experiments. Subcultures obtained from day 21 derive from mesangial cells (Foidart *et al.*, 1979; Kurtz *et al.*, 1983). The cells were grown in RPMI 1640 (Boehringer, Mannheim, Germany) supplemented with 10% fetal bovine serum (Boehringer), penicillin at 100 units/ml, streptomycin at 100 μg/ml (Boehringer) and bovine insulin at 0.66 units/ml (Sigma). Tissue flasks and dishes were kept at 37 °C in a humidified atmosphere in incubators in air/CO_2 (19:1).

$^{45}Ca^{2+}$ uptake

The culture medium was substituted by prewarmed HBS consisting of 20 mM-Hepes, 148 mM-NaCl, 5 mM-KCl, 2 mM-$CaCl_2$, 1 mM-$MgCl_2$, 6 mM-glucose, pH 7.35, supplemented with $^{45}Ca^{2+}$ (4 μCi/ml) and the culture dishes were placed on a heater to maintain a temperature of 37 °C. Verapamil was added where indicated at a final concentration of 10^{-5} M 5 min prior to the addition of the agonists. The vasoconstrictive agents were dissolved either separately or in combination with verapamil in HBS containing $^{45}Ca^{2+}$. OAG stock solution in dimethyl sulphoxide was diluted to 1% dimethyl sulphoxide with HBS containing 0.3 mg of bovine serum albumin (fatty acid free)/ml. This OAG solution was then briefly sonicated under N_2 before addition to the cells. The final dimethyl sulphoxide concentration was $< 0.1\%$. Controls were done with HBS containing the solvent only. At 15 s, 30 s, 60 s and 120 s after the addition of the agonists, buffer was withdrawn and the cells were quickly washed with 10 × 1 ml of ice-cold HBS containing 10 mM-$CaCl_2$. The high Ca^{2+} concentration (10 mM) was chosen in order to diminish Ca^{2+} efflux from the cells. The cells were lysed by the addition of 2 ml of 0.5 M-NaOH and the radioactivity, which represents the amount of intracellular $^{45}Ca^{2+}$, was counted in Aquasol 2 (New England Nuclear) in a β-scintillation counter. An aliquot was taken for protein determination. The influx of Ca^{2+} was determined from the initial rate of $^{45}Ca^{2+}$ uptake by the cells. This initial rate of $^{45}Ca^{2+}$ uptake was linear over the 120 s periods of measurement as estimated from linear-regression analysis.

Cell labelling

Medium was removed and the cells were incubated in MEM (Boehringer) supplemented with fatty acid-free bovine serum albumin (0.3 mg/ml) (Sigma) and [^{14}C]arachidonic acid (0.25 μCi/ml) for 24 h. For the determination of phosphoinositides, cells were labelled with carrier-free $^{32}P_i$ (100 μCi/ml) in phosphate-poor MEM (50 μM-P_i) containing 10% dialysed fetal bovine serum for 24 h. For the determination of the inositol phosphates, cells were prelabelled for 72 h with *myo*-[2-^{3}H]inositol (10 μCi/ml) in MEM free of inositol, containing 10% dialysed fetal bovine serum.

Lipid extraction and separation

After the prelabelling period the medium was replaced by fresh medium either with or without agonists. Incubations were terminated by rapid withdrawal of incubation medium and the addition of 1 ml of ice-cold methanol to the cells. The lipid extraction was done according to Bligh & Dyer (1959) with a final proportion of 2 ml of methanol, 2 ml of chloroform and 1.6 ml of water (containing 0.74% KCl, 0.04% $CaCl_2$ and 0.034% $MgCl_2$). After removal of the first chloroform extract, the remaining methanol/water phase was acidified with HCl (final concentration 0.01 M) and extracted twice with 2 ml of chloroform. The chloroform extracts were combined and dried in a rotation evaporator, dissolved in 200 μl of chloroform/methanol (2:1, v/v) and an aliquot was taken for t.l.c. Thin layer plates (pre-coated silica gel 60 with concentration zone, 0.25 mm thick) from Merck, Darmstadt, Germany were used throughout all experiments. Separation of the lipids was done as described (Pfeilschifter *et al.*, 1984). In brief, for the separation of neutral lipids, chromatographs were developed in one dimension using *n*-heptane/diethyl ether/acetic acid (75:25:4, by vol.) For separation of phospholipids, chromatographs were developed in one dimension using chloroform/methanol/acetic acid/water (100:30:35:3, by vol.) Polyphosphoinositides were separated on thin layer plates pretreated with 1% potassium oxalate containing 2 mM-EDTA using chloroform/methanol/4 M-NH_4OH (9:7:2, by vol.)

Pretreated t.l.c. plates were activated for 30 min at 115 °C prior to addition of lipid samples. Lipid standards were added as carriers and visualized by iodine staining. ^{32}P-labelled lipids were additionally localized by autoradiography. ^{14}C-labelled lipids were analysed with a TLC-Linear-Analyzer LB 2821 (Berthold, München, Germany). The detection efficiency for ^{14}C was about 5%. ^{32}P-labelled lipids were scraped off and counted by liquid scintillation in water (Čerenkov counting).

Extraction and determination of inositol phosphates

After the prelabelling period the medium was removed and the cells were rinsed several times to remove free [^{3}H]inositol. After this procedure cells were incubated for various times in 1 ml of MEM with or without agents. After the different times the medium was aspirated and the reactions were terminated by addition of 1 ml of 15% (w/v) trichloroacetic acid. The trichloroacetic acid was removed with diethyl ether. The final extract was neutralized and applied to anion-exchange columns containing 1 ml of Dowex 1 X8 (100–200 mesh, formate form; Serva, Heidelberg, Germany). Free inositol and the inositol phosphates were eluted sequentially by using (*a*) water (inositol), (*b*) 5 mM-disodium tetraborate/60 mM-sodium formate (glycerophosphoinositol), (*c*) 0.1 M-formic acid/0.2 M-ammonium formate (IP_1), (*d*) 0.1 M-formic acid/0.4 M-ammonium formate (IP_2) and (*e*) 0.1 M-formic acid/1.0 M-ammonium formate (IP_3), exactly as described by Berridge (1983). The identification of IP_2 and IP_3 was cross-checked by co-running of ^{32}P-labelled IP_2 and IP_3 prepared as described by Downes *et al.* (1982). Radioactivity was counted in Aquasol 2 (New England Nuclear) in a β-scintillation counter.

Prostaglandin and protein analysis

Culture dishes were washed twice with L-15 medium (Boehringer) and at the onset of the experiment 1 ml of fresh L-15 medium with or without agents was added followed by a 5 min incubation. The medium was then withdrawn, frozen in liquid N_2 and stored at −80 °C until

assay for prostaglandins. Verapamil was added where indicated at a final concentration of 10^{-5} M 5 min prior to the addition of the agonists. PGE_2 concentrations of the culture media were determined by radioimmunoassay for PGE_2 (New England Nuclear). Cells were dissolved in 0.5 M-NaOH and protein was determined according to the method of Lowry *et al.* (1951) with bovine serum albumin (Sigma) as standard.

Chemicals

Lipid standards, angiotensin II, noradrenaline, AGEPC, TPA, OAG and verapamil were obtained from Sigma. [1-^{14}C]Arachidonic acid, *myo*-[2-^{3}H]inositol, $^{45}CaCl_2$ and carrier-free $^{32}P_i$ were purchased from Amersham International. All other chemicals were from Merck, Darmstadt, Germany.

RESULTS

Vasoconstrictors stimulate PGE_2 synthesis in mesangial cells

Basal and agonist-stimulated PGE_2 synthesis is summarized in Table 1. In the basal state PGE_2 production was 2.0 ng/5 min per mg of protein. Addition of noradrenaline (10^{-5} M), angiotensin II (10^{-7} M), and AGEPC (10^{-8} M) increased the rate of PGE_2 production by 93, 132 and 166% respectively. The addition of the calcium channel blocker verapamil (10^{-5} M) abolished the vasoconstrictor-induced stimulation of PGE_2 synthesis in mesangial cells (Table 1). Verapamil blocked the angiotensin II (10^{-7} M)-induced PGE_2 synthesis with an IC_{50} value of 4×10^{-6} M. Verapamil alone had no effect on basal PGE_2 synthesis. Verapamil had also no effect on the PGE_2 synthesis evoked by the addition of exogenous arachidonic acid (results not shown). This clearly shows that verapamil did not interfere with cyclo-oxygenase activity.

Table 1. Effect of verapamil on basal and vasoconstrictor-stimulated PGE_2 synthesis

Mesangial cells were incubated and PGE_2 synthesis was measured as outlined under 'Experimental procedures'. Verapamil was added at a final concentration of 10^{-5} M 5 min prior to the addition of the agonists. Results are means ± S.E.M. for the number of experiments shown in parentheses.

Addition	PGE_2 synthesis (ng/5 min per mg of protein)
Control	2.00 ± 0.23 (12)
Verapamil (10 μM)	2.24 ± 0.16 (6)
Noradrenaline (10 μM)	3.86 ± 0.14 (5)
Noradrenaline (10 μM) + verapamil (10 μM)	1.98 ± 0.17 (5)
Angiotensin II (100 nM)	4.64 ± 0.32 (5)
Angiotensin II (100 nM) + verapamil (10 μM)	2.12 ± 0.20 (5)
AGEPC (10 nM)	5.32 ± 0.46 (5)
AGEPC (10 nM) + verapamil (10 μM)	1.89 ± 0.24 (5)

Table 2. Effect of verapamil on basal and vasoconstrictor-stimulated $^{45}Ca^{2+}$ uptake in mesangial cells

The cells were incubated and $^{45}Ca^{2+}$ influx was measured as outlined under 'Experimental procedures'. Verapamil was added at a final concentration of 10^{-5} M 5 min prior to the addition of the agonists. Results are means ± S.E.M. for the number of experiments shown in parentheses.

Addition	$^{45}Ca^{2+}$ influx (c.p.m./min per mg of protein)
Control	1648 ± 106 (10)
Verapamil (10 μM)	989 ± 100 (10)
Noradrenaline (10 μM) + verapamil (10 μM)	1039 ± 149 (5)
Angiotensin II (100 nM) + verapamil (10 μM)	1157 ± 118 (5)
AGEPC (10 nM) + verapamil (10 μM)	995 ± 131 (5)

Vasoconstrictors stimulate $^{45}Ca^{2+}$ uptake by mesangial cells

The uptake of $^{45}Ca^{2+}$ was measured 15, 30, 60 and 120 s after the addition of 4 μCi of $^{45}Ca^{2+}$/ml to the cells incubated in the presence of 2 mM-$CaCl_2$. The basal $^{45}Ca^{2+}$ influx as calculated from the slope of the regression lines was 1648 + 106 c.p.m./min per mg of cell protein. Addition of noradrenaline (10^{-5} M), angiotensin II (10^{-7} M) and AGEPC (10^{-8} M) at the same time as $^{45}Ca^{2+}$ increased the influx to 1930 ± 87, 2359 ± 212 and 2767 ± 238 c.p.m./min per mg of cell protein (means ± S.E.M. from five to ten experiments). Table 2 shows the effect of the calcium channel blocker verapamil (10^{-5} M) on the hormone-stimulated Ca^{2+} influx in mesangial cells. The stimulation of Ca^{2+} influx by noradrenaline, angiotensin II and AGEPC was decreased to 63, 70 and 60% of the control value, respectively. Verapamil alone decreased the Ca^{2+} uptake to 60% of the control value.

Vasoconstrictors stimulate phospholipase C in mesangial cells

Fig. 1 shows the time course of the decrease in radioactivity corresponding to PIP_2 in response to the three vasoconstrictive agents investigated. Angiotensin II (10^{-7} M), noradrenaline (10^{-5} M) and AGEPC (10^{-8} M) caused a rapid disappearance of ^{32}P label in PIP_2. This response was initiated rapidly and reached maximal values within 10 s for angiotensin II and noradrenaline and at 20 s for AGEPC. This rapid loss of radioactivity that occurred during the first 30 s or so of stimulation was followed by a recovery of the PIP_2 labelling towards its initial level, followed by an increase above this value at 2 min.

The data for PIP and PI are qualitatively similar to those for PIP_2 (not shown). The initial decrease of PIP, however, was not statistically significant and PIP levels rapidly increased above the initial values. After an initial lag phase the radioactivity in PI showed a significant decrease with a nadir at 20–30 s followed by a resynthesis with an overshoot in relation to the initial values. This appears to eliminate one possibility for the disappearance of ^{32}P label from PIP_2, namely its conversion into PIP by the specific phosphomonoesterase. If this reaction had

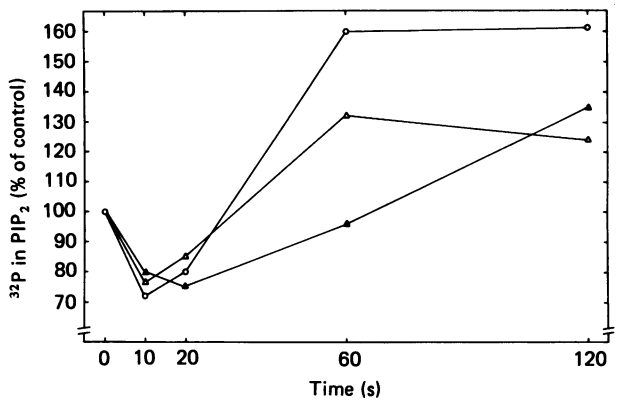

Fig. 1. Time course of vasoconstrictor-induced PIP_2 breakdown

$^{32}P_i$-labelled mesangial cells were stimulated with 10 μM-noradrenaline (△), 100 nM-angiotensin II (○), 10 nM-AGEPC (▲) or vehicle. Values are expressed as percentage of control and are the mean from five experiments; the S.E.M. varies from 5 to 16%.

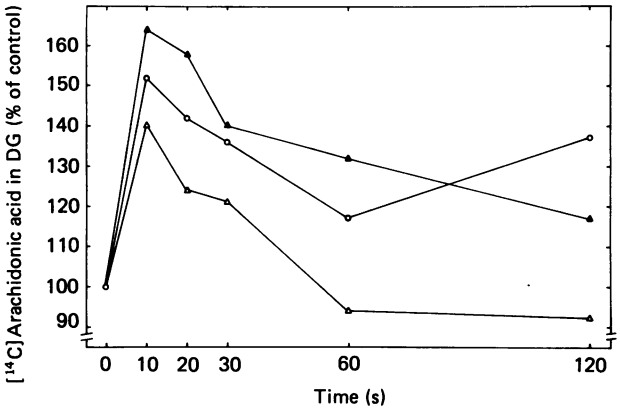

Fig. 2. Time course of vasoconstrictor-induced changes in [^{14}C]arachidonic acid radioactivity in DG

Mesangial cells prelabelled with [^{14}C]arachidonic acid were stimulated with 10 μM-noradrenaline (△), 100 nM-agiotensin II (○), 10 nM-AGEPC (▲) or vehicle. Values are expressed as percentage of control and are the means of five experiments; the S.E.M. ranges from 4 to 12%.

taken place it would have led to a rapid increase in PIP labelling, i.e. to the opposite of the observed results. The decrease in PI labelling is probably due to the increased resynthesis of PIP and PIP_2. However, we cannot rule out the possibility that phospholipase C also directly cleaves PI. The increase in PI labelling after 1 min is not easy to reconcile with a resynthesis of polyphosphoinositides from PI. In this instance one would expect a further decrease but not an increase of PI. One can speculate that there are different pools of PI, one of which is more readily available for resynthesis than the other. We have ascertained in independent experiments that the PI pool was in isotopic equilibrium. However some small pools of PI may have been below the detection level and these pools could have been the source for polyphosphoinositide resynthesis.

Simultaneously with the disappearance of ^{32}P-radioactivity from PIP_2 there was an increase of label in DG. Addition of angiotensin II, noradrenaline and AGEPC to mesangial cells prelabelled with [^{14}C]arachidonic acid stimulated a rapid and transient formation of [^{14}C]DG (Fig. 2). The maximal increase of label in DG could be observed as early as 10 s after addition of the vasoconstrictors and reaches 152%, 140% and 164% of control level for angiotensin II, noradrenaline and AGEPC, respectively. Thereafter the values for ^{14}C radioactivity in DG decreased towards control levels.

Stimulation of mesangial cells with angiotensin II, noradrenaline and AGEPC also resulted in significant increases in the levels of IP_1, IP_2 and IP_3 as early as 10 s after the addition of the agonists, as shown in Table 3. Simultaneous addition of verapamil and noradrenaline did not alter the rate of activation nor the time course of activation of phospholipase C. Table 4 shows that verapamil had no effect on the degradation of PIP_2 and the production of DG and IP_3 caused by the addition of noradrenaline. Verapamil alone did not alter the levels of PIP_2, DG and IP_3 in mesangial cells at the time point examined.

Table 3. Effect of vasoconstrictors on inositol phosphate accumulation in mesangial cells

Cultured mesangial cells were prelabelled with *myo*-[2-^{3}H]inositol for 72 h as described under 'Experimental procedures'. The cells were then stimulated for 10 s with 10 μM-noradrenaline, 100 nM-angiotensin II and 10 nM-AGEPC. Values are expressed as means ± S.E.M. obtained from five independent experiments.

Addition	[^{3}H]radioactivity (c.p.m./mg of protein) in: IP_1	IP_2	IP_3
Control	393 ± 37	113 ± 22	75 ± 10
Noradrenaline	962 ± 64	352 ± 38	141 ± 17
Angiotensin II	1061 ± 72	395 ± 52	163 ± 15
AGEPC	1084 ± 104	384 ± 63	175 ± 22

Table 4. Effect of verapamil on noradrenaline-induced activation of phospholipase C

Mesangial cells were prelabelled with $^{32}P_i$, [^{14}C]arachidonic acid or *myo*-[2-^{3}H]inositol respectively, as described under 'Experimental procedures'. The cells were then stimulated for 10 s with 10 μM-noradrenaline, 10 μM-noradrenaline plus 10 μM-verapamil or vehicle. Radioactivity in PIP_2, DG and IP_3 respectively was determined as outlined under 'Experimental procedures'. Values are the means of five experiments ± S.E.M.

Addition	Radioactivity (c.p.m./mg of protein) in: PIP_2	DG	IP_3
Control	28650 ± 1720	50 ± 5	75 ± 10
Noradrenaline	21790 ± 1340	80 ± 7	141 ± 17
Noradrenaline + verapamil	19850 ± 1920	74 ± 9	152 ± 19

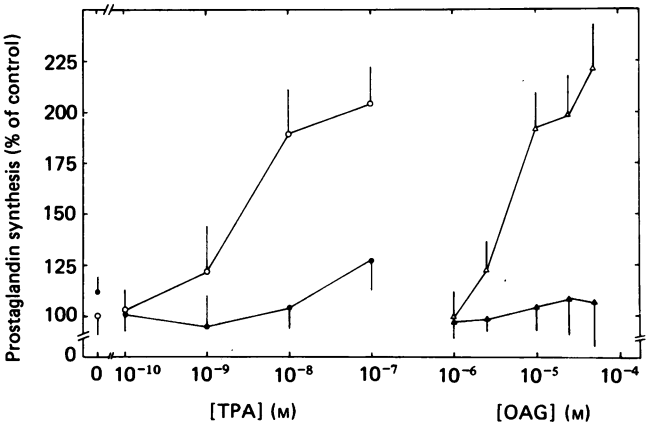

Fig. 3. Dose–response curve to TPA and OAG for PGE_2 synthesis in mesangial cells

The effect of TPA (○, ●) and OAG (△, ▲) on PGE_2 synthesis in mesangial cells is shown; ●, ▲, the effect of 10 μM-verapamil on this stimulated PGE_2 synthesis. Values are the mean of seven experiments ± S.E.M.

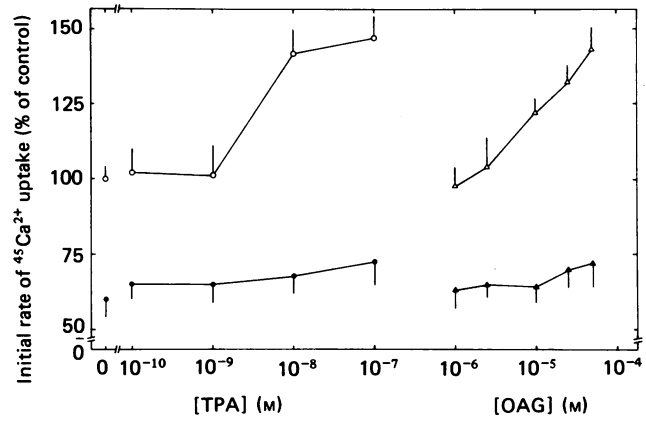

Fig. 4. Dose–response curve to TPA and OAG for initial uptake of $^{45}Ca^{2+}$ into mesangial cells

The effect of TPA (○, ●) and OAG (△, ▲) on initial uptake of $^{45}Ca^{2+}$ into mesangial cells is shown; ●, ▲, the effect of 10 μM-verapamil on this stimulated $^{45}Ca^{2+}$ uptake. Values are the mean of seven experiments ± S.E.M.

Effect of phorbol esters and OAG on PGE_2 synthesis and $^{45}Ca^{2+}$ uptake in mesangial cells

Noradrenaline, angiotensin II as well as AGEPC increase the concentration of DG in mesangial cells. We therefore wanted to test whether the activation of protein kinase C by DG would be a common mechanism by which vasoconstrictors enhance calcium permeability of the cell membrane and provoke PG synthesis in mesangial cells. Like DG, TPA is also known to increase greatly the affinity of protein kinase C for calcium, thereby leading to an activation of the enzyme (Kishimoto *et al.*, 1980). We therefore examined the influence of the phorbol esters TPA and 4α-phorbol 12,13-didecanoate as well as the synthetic DG analogue OAG on PG synthesis and $^{45}Ca^{2+}$ uptake. Figs. 3 and 4 show that TPA and OAG increased PG synthesis and enhanced $^{45}Ca^{2+}$ uptake in a dose-dependent manner which was also observed with angiotensin II, noradrenaline and AGEPC. In further analogy with the vasoconstrictive hormones, the effects of TPA and OAG on calcium uptake and PG synthesis could be inhibited by verapamil (10^{-5} M). Chelation of calcium with EDTA also inhibited TPA- and OAG-stimulated PGE_2 synthesis. The non-tumour-promoting 4α-phorbol 12,13-didecanoate at a concentration of 10^{-7} M did not increase PGE_2 synthesis nor $^{45}Ca^{2+}$ uptake in mesangial cells. TPA itself up to concentration of 10^{-7} M did not alter the levels of the inositol phosphates or DG during the first 2 min after its addition to mesangial cells.

DISCUSSION

It was the aim of the present study to obtain information on the possibility of a common mechanism by which the vasoconstrictors angiotensin II, noradrenaline and AGEPC increase PG synthesis in renal mesangial cells. All three vasoconstrictors significantly enhanced PG production (Table 1) and $^{45}Ca^{2+}$ uptake by mesangial cells. Both effects could be abolished by the calcium channel blocker verapamil (Tables 1 and 2). Since calcium influx into cells is thought to be limited by the availability of open calcium channels in the plasma membrane, the question arises as to which mechanism angiotensin II, noradrenaline and AGEPC stimulate calcium influx, thereby increasing PG synthesis. In general, hormones that mediate physiological responses through calcium mobilization also stimulate the metabolism of membrane phosphoinositides (cf. Michell *et al.*, 1981; Michell, 1982). This was also found to be true of angiotensin II, noradrenaline and AGEPC in cultured mesangial cells. The results presented in Figs. 1 and 2 and in Table 3 clearly indicate that these vasoconstrictors caused an activation of phospholipase C, which led to an increased formation of two important second messengers, IP_3 and DG. There is growing evidence that DG and IP_3 synergistically mediate signal transduction in receptor systems linked to calcium mobilization (cf. Berridge, 1984). IP_3 is thought to be the intracellular signal to promote hormone-induced calcium mobilization (cf. Berridge & Irvine, 1984) and DG activitates protein kinase C which in turn phosphorylates various cellular proteins (cf. Nishizuka, 1984). In mesangial cells it seems that the calcium released by IP_3 is either not sufficient to increase PG formation over basal values, or is being sequestered intracellularly in such a way that it has no access to Ca^{2+}-regulated phospholipases which cleave off arachidonate. This can be inferred from the finding that verapamil inhibited vasoconstrictor-induced influx of $^{45}Ca^{2+}$ on PG synthesis but had no effect whatsoever on the formation of IP_3 (Table 4). Theoretically it is possible that verapamil not only inhibits Ca^{2+} entry across the plasma membrane but also IP_3-induced Ca^{2+} release from intracellular stores. The available experimental evidence, however, argues against this latter possibility (Biden *et al.*, 1984).

The increase of the Ca^{2+} permeability of the plasma membrane upon challenging mesangial cells with vasoconstrictive hormones is likely to be related to a stimulation of protein kinase C by DG. This contention receives experimental support from the finding that TPA and OAG enhanced PG synthesis and $^{46}Ca^{2+}$ entry in a dose-dependent manner and that these effects could again

be blocked by verapamil or by omission of extracellular calcium (Figs. 3 and 4). TPA and OAG are potent activators of protein kinase C (Castagna *et al.*, 1982; Nishizuka, 1983) which are widely used to by-pass hormone-induced alterations of the phosphoinositide metabolism of the cell membrane. In contrast, 4α-phorbol 12,13-didecanoate, a phorbol ester which does not stimulate protein kinase C (Nishizuka, 1984), had no effect whatsoever on either Ca^{2+} entry or PG synthesis.

This result renders an unspecific effect of OAG and TPA on the cell metabolism unlikely and further supports the role of protein kinase C in regulating the Ca^{2+} permeability of the plasma membrane of renal mesangial cells.

It is noteworthy in this connection that TPA stimulates PG synthesis in different tissues (Levine & Hassid, 1977; Butler-Gralla *et al.*, 1983; Snoek & Levine, 1983; Kucera *et al.*, 1984) including cultured MDCK cells. The TPA-induced synthesis in MDCK cells was attributed to an activation of phospholipase A_2 (Daniel *et al.*, 1981; Beaudry *et al.*, 1983) which is known to be activated by Ca^{2+}. Our results on the regulation of Ca^{2+} permeability by protein kinase C offer an explanation for the results obtained in MDCK cells. In view of the fact that mesangial cells are the contractile elements of the renal glomeruli (Scharschmidt & Dunn, 1983; Fujiwara *et al.*, 1984) it is particularly interesting that Rasmussen *et al.* (1984) and Danthuluri & Deth (1984) were able to produce Ca^{2+}-dependent contraction of arterial vessel preparations with TPA.

These results, taken together with the observations reported in the present study, point towards a more general role of DG-mediated activation of protein kinase C in the recruitment of extracellular calcium. Exactly by which mechanisms this physiological regulation is being effected is unknown. In analogy with cyclic AMP-dependent protein kinases (Kandel & Schwartz, 1982; Nestler & Greengard, 1983), the phosphorylation of parts of a calcium channel could be a possibility for the mode of action of the protein kinase C as well.

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