Biochemical Pharmacology, Vol. 37, No. 21, pp. 4205–4210, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

CYCLOSPORIN A AUGMENTS VASOCONSTRICTOR-INDUCED RISE IN INTRACELLULAR FREE CALCIUM IN RAT RENAL MESANGIAL CELLS

JOSEF PFEILSCHIFTER*
Physiologisches Institut der Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

(*Received* 21 *January* 1988; *accepted* 7 *June* 1988)

Abstract—Pretreatment of rat renal mesangial cells with the immunosuppressive drug cyclosporin A caused a dose-dependent increase in the angiotensin II, [Arg^8]vasopressin and noradrenalin-stimulated rise in intracellular free calcium as measured with quin 2. Cyclosporin A had no significant effect on basal cytosolic free calcium. However, cyclosporin A increased the basal $^{45}Ca^{2+}$ influx. This stimulated $^{45}Ca^{2+}$ influx was not blocked by nifedipine (10^{-6} M). Cyclosporin A also augmented the angiotensin II, [Arg^8]vasopressin and noradrenalin-stimulated efflux of $^{45}Ca^{2+}$ from mesangial cells. These results suggest that cyclosporin A stimulates transmembrane Ca^{2+} influx in mesangial cells and also augments the vasoconstrictor-induced increases in cytosolic free calcium.

Cyclosporin A (CsA)† is a potent immunosuppressive drug which is widely used in patients with organ transplantation and in patients with autoimmune diseases [1–3]. However, the use of CsA is hampered by a number of side effects, with nephrotoxicity being the most common and serious one [4–6]. CsA-induced nephrotoxicity is characterized by a reversible reduction in glomerular filtration rate and renal blood flow as well as by tubular and interstitial damage [7–9]. The local modulation of glomerular filtration depends on a subtle balance between the actions of circulating agents on mesangial cells and of locally generated autocoids such as prostaglandins [10, 11]. Renal mesangial cells are considered to be modified smooth muscle cells, as they contract when challenged with vasoactive agents and thereby reduce the glomerular ultrafiltration coefficient, K_f [10, 11]. Recently it was shown that CsA augments the angiotensin II-stimulated increases in intracellular free calcium in vascular smooth muscle cells [12]. This effect of CsA to enhance vasoconstrictor-mediated calcium mobilization in smooth muscle cells was suggested to be a possible causal factor in CsA-induced hypertension. The present investigation was designed to determine if CsA alters vasoconstrictor-evoked calcium mobilization in rat renal mesangial in a way similar to that described for vascular smooth muscle cells and might thereby contribute to CsA nephrotoxicity.

MATERIALS AND METHODS

Cell culture. Cultivation of rat mesangial cells was done as described [13]. For all experiments passages 1–3 of mesangial cells were used. The cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and bovine insulin at 0.66 unit/ml (Sigma). Mesangial cells were characterized by different criteria such as morphology in phase contrast microscope, prominent staining for the intermediate filament desmin, which is considered to be specific for myogenic cells [14] and growth in D-valine containing medium [15].

Measurement of $^{45}Ca^{2+}$ influx and $[Ca^{2+}]_i$. Influx of $^{45}Ca^{2+}$ was measured 15, 45, 75 and 105 sec after the addition of 1 ml HBS, consisting of 20 mM Hepes, 148 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 6 mM glucose, pH 7.35, supplemented with 4 μCi of $^{45}Ca^{2+}$ to cells in 35 mm diameter dishes. Calculations were done as described previously [16]. $[Ca^{2+}]_i$ was measured using the fluorescent calcium indicator quin 2 as described [17]. In brief, approx. 2×10^7 cells were incubated with 25 μM quin 2/AM in RPMI 1640 medium for 20 min followed by another 40 min incubation with 4 vol. of the medium. Aliquots of 10^6 cells were washed twice and resuspended in HBS containing 1.3 mM Ca^{2+}. Fluorescence of quin 2-loaded cells was measured at 37° in a Shimadzu RF-510 spectrofluorophotometer using an excitation wave length of 340 nm and an emission wavelength of 492 nm. The fluorescence signal was calibrated at the end of each individual trace as described by Tsien *et al.* [18]. In order to establish the influence of extracellular Ca^{2+} on the angiotensin II-induced increases in free cytoplasmic Ca^{2+}, cell suspensions were treated with 1.3 mM EGTA to chelate Ca^{2+} or with the calcium channel blocker nifedipine (1 μM) 1 min prior to treatment with angiotensin II.

$^{45}Ca^{2+}$ efflux assay. Cells cultured in 16 mm diameter wells were incubated for 24 hr at 37° in RPMI 1640 containing 10% fetal calf serum plus 4 μCi of $^{45}Ca^{2+}$/ml to ensure complete labelling of exchange-

* Present address: Ciba-Geigy Ltd., Pharmaceutical Research Division, R-1056.P.23, CH-4002 Basel, Switzerland.

† Abbreviations used: CsA, cyclosporin A; $[Ca^{2+}]_i$, intracellular free calcium concentration; HBS, hepes-buffered saline.

able pools of Ca^{2+}. Aliquots of CsA solution or vehicle were then added and the cells were incubated for 1 hr. Efflux experiments were initiated by rapidly washing the cells four times with HBS at 37° to remove extracellular $^{45}Ca^{2+}$ and by adding 0.5 ml HBS. Efflux was measured for 3 × 5 min and was found to be linear after this period. Angiotensin II (10^{-7} M), [Arg^8]vasopressin (10^{-7} M), noradrenalin (10^{-5} M) or vehicle was added and efflux was measured for further 3 × 5 min. The incubation was terminated by washing the cells in ice-cold HBS followed by addition of 0.1% SDS. An aliquot was counted to determine the $^{45}Ca^{2+}$ content remaining in the cells. The average loss of $^{45}Ca^{2+}$ (expressed as fractional loss/5 min) during a given sampling interval was calculated: fractional loss = (cellular $^{45}Ca_{t1}$ − cellular $^{45}Ca_{t2}$)/(cellular $^{45}Ca_{t1}$) × ($t_2 - t_1$).

Chemicals. Angiotensin II [Arg^8]vasopressin, noradrenalin and nifedipine were from Sigma (St Louis, MO), cyclosporin A was a generous gift from Dr U. T. Rüegg from Sandoz, (Basel, Switzerland) $^{45}CaCl_2$ from Amersham International (Bucks, U.K.) quin 2/AM from Calbiochem, (Luzern, Switzerland) cell culture media and nutrients were from Boehringer-Mannheim (F.R.G) and all other chemicals were from Merck (Darmstadt, F.R.G.).

RESULTS

Effects of CsA *on vasoconstrictor-stimulated rises in* $[Ca^{2+}]_i$

The basal $[Ca^{2+}]_i$ as measured with the fluorescent calcium indicator quin 2 was 129 ± 28 nM (mean ± SEM, N = 14). Angiotensin II (10^{-7} M) increased this transiently to 209 ± 19 nM (mean ± SEM, N = 5). Pretreatment of the cells with CsA had no effect on basal $[Ca^{2+}]_i$; which was calculated to be 134 ± 17 nM for 2 μg CsA/ml and 127 ± 15 nM for 10 μg CsA/ml (mean ± SEM, N = 5). However, CsA dose-dependently increased the peak value of angiotensin II-stimulated increase in cytosolic free calcium and delayed the decay observed thereafter (Fig. 1). Preincubation with CsA also enhanced the Ca^{2+} mobilization evoked by [Arg^8]vasopressin (10^{-7} M) and noradrenalin (10^{-5} M) as shown in Table 1, suggesting that this effect of CsA on intracellular calcium homeostasis is a more general phenomenon. In order to establish whether the angiotensin II-induced rise in $[Ca^{2+}]_i$ requires the presence of extracellular Ca^{2+}, cell suspensions were treated with 1.3 mM-EGTA to chelate Ca^{2+} prior to addition of the hormone. Under these conditions, angiotensin II (10^{-7} M) elicited a rise in cytosolic free Ca^{2+} that was only slightly reduced in its peak value and returned back to basal values more rapidly than in the presence of normal extracellular calcium concentrations (Fig. 2). Again pretreatment of mesangial cells with CsA dose-dependently enhanced the $[Ca^{2+}]_i$-transients in response to angiotensin II as shown in Fig. 2. This indicates that CsA enhanced the angiotensin II-stimulated mobilization of Ca^{2+} from intracellular stores. However, the peak values of $[Ca^{2+}]_i$; in cells pretreated with CsA were significantly lower in the presence of EGTA as compared to cells with normal extracellular Ca^{2+} concentration (Table 2). This indicates that CsA not only increases the hormone-induced mobilization of Ca^{2+} from intracellular stores but also increases the influx of Ca^{2+} from the extracellular space. To further characterize the angiotensin II-evoked Ca^{2+} mobilization the dihydropyridine Ca^{2+} channel blocker nifedipine was tested. Pretreatment of mesangial cells with nifedipine (10^{-6} M) had no influence on angiotensin II-induced rises in $[Ca^{2+}]_i$ either in control cells or in cells treated with CsA as

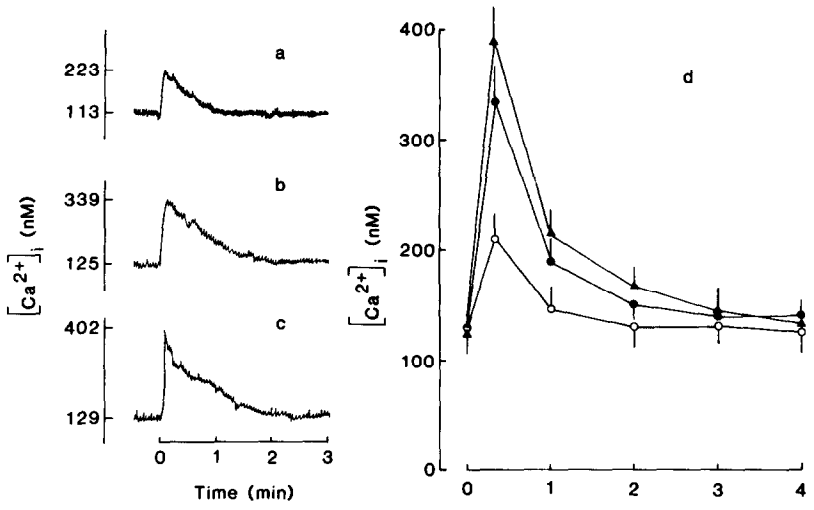

Fig. 1. Effects of CsA treatment on the angiotensin II-induced rise in $[Ca^{2+}]_i$. Fluorescence of quin 2 loaded mesangial cells was registered. At time point 0 angiotensin II (10^{-7} M) was added to the cells. (a)–(c) Individual fluorescence traces: (a) control cells, (b) cells pretreated with CsA (2 μg/ml) for 1 hr, (c) cells pretreated with CsA (10 μg/ml) for 1 hr. (d) Mean values of $[Ca^{2+}]_i$ from 5–14 experiments: ○, control cells; ●, cells pretreated with CsA (2 μg/ml) for 1 hr; ▲, cells pretreated with CsA (10 μg/ml) for 1 hr.

Table 1. Effect of CsA treatment on the [Arg^8]vasopressin and noradrenalin-induced rise in $[Ca^{2+}]_i$

Addition	Peak value of $[Ca^{2+}]_i$ (nM)
Control	132 ± 19*
CsA (2 μg/ml)	131 ± 14
[Arg^8]vasopressin (10^{-7}M)	273 ± 24
[Arg^8]vasopressin (10^{-7} M) + CsA (10 μg/ml)	399 ± 31
Noradrenalin (10^{-5}M)	218 ± 20
Noradrenal (10^{-5}M) + CsA (10 μg/ml)	324 ± 27

Fluorescence of quin 2 loaded mesangial cells was registered. Peak values for [arg^8]vasopressin (10^{-7}M) and noradrenalin (10^{-5}M)-stimulated rise in $[Ca^{2+}]_i$ are indicated in control cells and cells pretreated with CsA (10 μg/ml) for 1 hr. Results are means ± SEM for 3–6 experiments.

* This value is calculated from 6 experiments done together with the determination of vasopressin and noradrenalin effects and therefore differs from the value indicated on the previous page. (129 ± 28 nM).

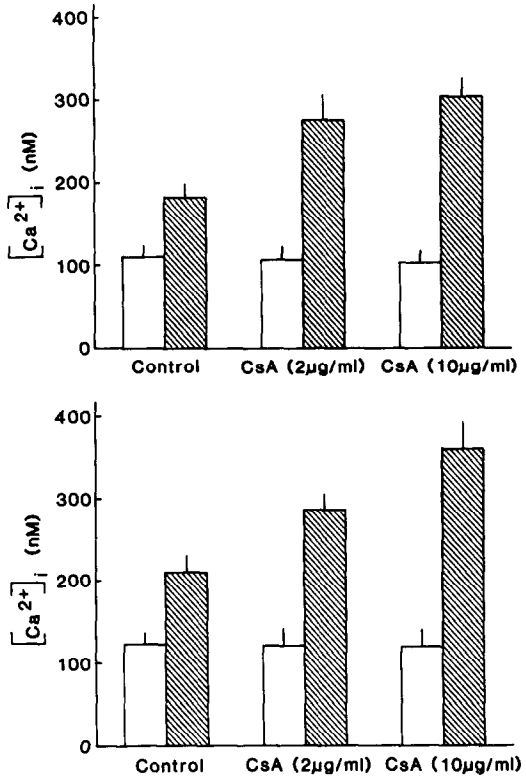

Fig. 2. Effect of EGTA and nifedipine on angiotensin II-induced rise in $[Ca^{2+}]_i$; in control cells and cells treated with CsA. Upper panel: cells were loaded with quin 2 and EGTA 1.3 mM was added 1 min before the addition of angiotensin II (10^{-7}M). Lower panel: cells were loaded with quin 2 and nifedipine (10^{-6} M) was added 1 min before the addition of angiotensin II (10^{-7} M). Open bars indicate basal levels of $[Ca^{2+}]_i$; hatched bars indicate the peak values of the stimulated rises in $[Ca^{2+}]_i$ for control cells and cells pretreated for 1 hr with the indicated concentrations of CsA. Results are means ± SEM for 3 experiments. The differences in $[Ca^{2+}]_i$ between control plus angiotensin II versus CsA plus angiotensin II are significant for cells pretreated with EGTA as well as for cells pretreated with nifedipin ($P < 0.01$ for CsA (2 μg/ml) and $P < 0.005$ for CsA (10 μg/ml) by Student's *t*-test).

shown in Fig. 2 and Table 2. This indicates that Ca^{2+}-influx via L-type Ca^{2+} channels does not contribute to the angiotensin II-evoked Ca^{2+} transient in mesangial cells.

Effects of CsA *on vasoconstrictor-evoked transmembrane* $^{45}Ca^{2+}$ *fluxes*

To strengthen further the suggestion that CsA augments the hormone-stimulated Ca-release from intracellular stores the $^{45}Ca^{2+}$ efflux from mesangial cells was measured. In the absence of any agonist, a nearly constant amount of $^{45}Ca^{2+}$ was released from the cells in each 5 min period. Addition of angiotensin II (10^{-7} M), [Arg^8]vasopressin (10^{-7} M) or noradrenalin (10^{-5} M) increased the amount of $^{45}Ca^{2+}$ appearing in the external medium as shown in Table 3. Pretreatment of mesangial cells with CsA had no effect on the basal release of $^{45}Ca^{2+}$ but augmented the vasoconstrictor-evoked $^{45}Ca^{2+}$ efflux. As efflux is a measure of the hormone-induced release of intracellular calcium, these results also argue that CsA treatment enhances the vasoconstrictor-stimulated mobilization of Ca^{2+} from intracellular stores. Furthermore, these results exclude the possibility that the increased amplitude and duration of the hormone-stimulated increases in $[Ca^{2+}]_i$ were due to an inhibition of the Ca^{2+} extrusion mechanisms in mesangial cells.

Table 2. Effect of EGTA and nifedipine an angiotensin II-stimulated rise in $[Ca^{2+}]_i$ in CsA pretreated cells

Addition	Peak value of $[Ca^{2+}]_i$ (nM)	Number of experiments	P-value (vs control)
Control	335 ± 31	5	—
EGTA	275 ± 29	3	< 0.05
Nifedipine	287 ± 19	3	n.s.

Fluorescence of quin 2 loaded cells was registered. Peak values for angiotensin II (10^{-7}M)-stimulated rise in $[Ca^{2+}]_i$ are indicated for cells pretreated with CsA (2 μg/ml) for 1 hr. EGTA (1.3 mM) or nifedipine (10^{-6}M) were added 1 min prior to the addition of angiotensin II.

P-values were calculated by Student's *t*-test.

n.s.: not significant.

Table 3. Effect of CsA on basal and angiotensin II, [Arg^8]vasopressin and noradrenalin-stimulated $^{45}Ca^{2+}$ efflux from renal mesangial cells

Addition	Fractional loss of $^{45}Ca^{2+}$
Control	0.10 ± 0.01
Angiotensin II (10^{-7} M)	0.34 ± 0.02
[Arg^8]vasopressin (10^{-7} M)	0.32 ± 0.03
Noradrenalin (10^{-5} M)	0.24 ± 0.02
CsA (10 μg/ml)	0.11 ± 0.02
Angiotensin II (10^{-7} M) + CsA (10 μg/ml)	0.46 ± 0.03*
[Arg^8]vasopressin (10^{-7} M) + CsA (10 μg/ml)	0.47 ± 0.04*
Noradrenalin (10^{-5} M) + CsA (10 μg/ml)	0.34 ± 0.03*

The cells were prelabelled with $^{45}Ca^{2+}$ (4 μCi/ml) for 24 hr and CsA (10 μg/ml) was then added for a further 1 hr. Cells were then stimulated with angiotensin II (10^{-7} M), [Arg^8]vasopressin (10^{-7} M), noradrenalin (10^{-5} M) or vehicle. Data are expressed as fractional loss/5 min. The values for the first interval after the addition of angiotensin II, [Arg^8]vasopressin, noradrenalin or vehicle are indicated. Results are means ± SEM for 6 experiments.

* $P < 0.05$ versus the different hormones alone, by Student's t-test.

In smooth muscle cells we have shown that CsA increases basal $^{45}Ca^{2+}$ influx and this may increase the hormone-sensitive intracellular Ca^{2+} stores [12]. As compared to control cells, CsA increased $^{45}Ca^{2+}$ uptake also in mesangial cells (Table 4). Basal $^{45}Ca^{2+}$ uptake was stimulated by 34% when mesangial cells were pretreated with 10 μg/ml CsA for 1 hr.

DISCUSSION

A reversible reduction in glomerular filtration rate is a frequent and serious side-effect in patients treated with CsA. The mechanism of acute nephrotoxicity of CsA, however, is not well understood. The exquisite localization of mesangial cells in the intercapillary space of the glomerulum, and its ability to respond to vasoactive hormones emphasize the importance of this cell type in the maintenance of a well regulated glomerular filtration rate. Mesangial cells are a target for CsA and it has been shown that CsA suppresses the proliferation of mesangial cells [19]. In this report it is demonstrated that CsA augments the Ca^{2+} mobilization in mesangial cells stimulated with the vasoconstrictive hormones angiotensin II, [Arg^8]vasopressin and noradrenalin. As mesangial cells are modified smooth muscle cells and contract upon challenge with the above mentioned vasoconstrictive hormones, an augmented and prolonged increase in $[Ca^{2+}]_i$ may result in an enhanced constrictory response of the cells. This may cause an amplified reduction in the glomerular ultrafiltration coefficient, K_f, and hence a reduction in the glomerular filtration rate. The contractile effects of angiotensin II and vasopressin on mesangial cells are normally attenuated by an increased formation of vasodilatory prostaglandins (PGE_2, PGI_2) [10]. In a recent report it has been shown that CsA inhibits angiotensin II-stimulated prostaglandin E_2 generation by mesangial cells [20]. Similar results were reported for vascular smooth muscle cells [21] and it has been concluded that CsA acts by inhibiting phospholipase A activity. Thus CsA may cause an amplified reduction of K_f by a dual attack: first, an increased Ca^{2+} mobilization by vasoconstrictive hormones with a subsequent constrictory response and second, an attenuated formation of vasodilatory prostaglandins with a subsequent lack of modulation of the contractile state of the mesangial cells. However, one has to be aware that the concentrations of the tested vasoactive agents are rather high when compared to the physiologically occurring concentration of the hormones.

Table 4. Effect of CsA on basal $^{45}Ca^{2+}$ influx in mesangial cells

Addition	$^{45}Ca^{2+}$ influx (cpm/min per mg of protein)
Control	1783 ± 132
CsA (10 μg/ml)	2391 ± 143*

The cells were incubated with CsA (10 μg/ml) or vehicle for 1 hr and $^{45}Ca^{2+}$ influx was then measured as outlined under Materials and Methods. Results are means ± SEM for 6 experiments.

* $P < 0.05$ versus control, by Student's t-test.

The intracellular mechanism of CsA effects on target cells is unclear. A cytosolic binding protein for CsA named cyclophilin [22] and CsA binding to calmodulin ([23], but see also [24]) have been suggested as possible mediators of CsA action. Angiotensin II, [Arg^8]vasopressin and noradrenalin activate a phospholipase C in mesangial cells which specifically cleaves phosphatidylinositol 4,5-bisphosphate to yield diacylglycerol and inositol 1,4,5-trisphosphate [13, 16, 17]. Inositol 1,4,5-trisphosphate is the second messenger for the mobilization of Ca^{2+} from intracellular stores in various cell types (see Ref. 25 for review) including mesangial cells [17, 26]. A promotion of the accumulation of inositol 1,4,5-trisphosphate could be a possible mechanism for explaining the observed CsA-induced increase in calcium mobilization in vasoconstrictor-stimulated

mesangial cells. An attenuated metabolism of inositol 1,4,5-trisphosphate would result in an increased mobilization of Ca^{2+} from intracellular stores and could therefore account for the observed increase in $^{45}Ca^{2+}$ efflux. As inositol 1,4,5-trisphosphate alone or in co-operation with inositol 1,3,4,5-tetrakisphosphate has been suggested to regulate Ca^{2+} influx [27, 28] an altered metabolism of these messengers might also account for the increased ^{45}Ca-influx in mesangial cells. There are two distinct mechanisms for terminating the calcium releasing action of inositol 1,4,5-trisphosphate. The first is a 5-phosphomonoesterase, which dephosphorylates inositol 1,4,5-trisphosphate to inositol 1,4-bisphosphate. The second pathway of metabolism of inositol 1,4,5-trisphosphate is the phosphorylation catalyzed by a 3-kinase to yield inositol 1,3,4,5-tetrakisphosphate (see Ref. 29 for review). As calmodulin activates the latter enzyme in pig aortic smooth muscle [30], CsA might interfere with the activity of this enzyme and thereby alter the metabolism of inositol 1,4,5-trisphosphate. Alternatively, cyclosporin A might inhibit the 5-phosphomonoesterase either directly or indirectly by inhibiting protein kinase C, as protein kinase C has been shown to enhance the catalytic activity of 5-phosphomonoesterase [31]. Preliminary experiments show that CsA does not inhibit protein kinase C partially purified from mesangial cells (J. Pfeilschifter, unpublished results) and therefore makes the latter possibility less probable. To prove the importance of the reported effects of CsA on Ca^{2+} homeostasis in mesangial cells and to unveil the exact mechanism of CsA action remains a task for future research.

Acknowledgements—The author gratefully acknowledges Mrs. G. Dervisoğlu for typing the manuscript and Mr. W. Gehret for doing the art work.

REFERENCES

1. Borel JF, Cyclosporine A present experimental status. *Transplant Proc* **13**: 344–348, 1981.
2. Kahan BD, Cyclosporine: The agent and its action. *Transplant Proc* **17**: 5–18, 1985.
3. Thomson AW, Whiting PH and Simpson JG, Cyclosporine: Immunology, toxicity and pharmacology in experimental animals. *Agents Actions* **15**: 306–327, 1984.
4. Flechner SM, van Buren G, Herman RH and Kahan BD, The nephrotoxicity of cyclosporine in renal transplant recipients. *Transplant Proc* **15**: 2689–2694, 1983.
5. Hows JM, Smith JM, Baughan A and Gordon-Smith EC, Nephrotoxicity in marrow graft recipients treated with cyclosporine. *Transplant Proc* **15**: 2708–2711, 1983.
6. Meyers BD, Cyclosporine nephrotoxicity. *Kidney Int* **30**: 964–974, 1986.
7. Murray BM, Paller MS and Ferris TF, Effect of cyclosporine administration on renal hemodynamics in conscious rats. *Kidney Int* **28**: 767–774, 1985.
8. Barros EJG, Boim MA, Ajzen H, Ramos OL and Schor N, Glomerular hemodynamics and hormonal participation on cyclosproine nephrotoxicity. *Kidney Int* **32**: 19–25, 1987.
9. Ryffel B, Siegl H, Thiel G and Mihatsch MJ, Experimental cyclosporine nephrotoxicity. In: *Kidney Transplant Rejection* (Eds. Williams GM, Burdick JF and Solez K), pp. 383–410. Marcel Dekker, New York, 1986.
10. Dworkin LD, Ichikawa I and Brenner BM, *Am J Physiol* **244**: F95–F104, 1983.
11. Schlondorff D, The glomerular mesangial cell: an expanding role for a specialized pericyte. *FASEB J* **1**: 272–281, 1987.
12. Pfeilschifter J and Rüegg UT, Cyclosporin A augments angiotensin II-stimulated rise in intracellular free calcium in vascular smooth muscle cells. *Biochem J* **248**: 883–887, 1987.
13. Pfeilschifter J, Kurtz A and Bauer C, Activation of phospholipase C and prostaglandin synthesis by (arginine)vasopressin in cultures of rat renal mesangial cells. *Biochem J* **223**: 855–859, 1984.
14. Travo P, Weber K and Osborn M, Co-existence of vimentin and desmin type intermediate filaments in a subpopulation of adult rat vascular smooth muscle cells growing in primary culture. *Exp Cell Res* **139**: 87–94, 1982.
15. Kreisberg JI and Karnovsky MJ, Glomerular cells in culture. *Kidney Int* **23**: 439–447, 1983.
16. Pfeilschifter J, Kurtz A and Bauer C, Role of phospholipase C and protein kinase C in vasoconstrictor-induced prostaglandin synthesis in cultured rat renal mesangial cells. *Biochem J* **234**: 125–130, 1986.
17. Pfeilschifter J and Bauer C, Pertussis toxin abolishes angiotensin II-induced phosphoinositide hydrolysis and prostaglandin synthesis in rat renal mesangial cells. *Biochem J* **236**: 289–294, 1986.
18. Tsien RY, Pozzan T and Rink TJ, Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with as new intracellularly trapped fluorescence indicator. *J Cell Biol* **94**: 325–334, 1982.
19. Martin M, Krichbaum M, Kaever V, Goppelt-Strübe M and Resch K, Cycylosporin A suppresses proliferation of renal mesangial cells in culture. *Biochem Pharmacol* **37**: 1083–1088, 1988.
20. Stahl RAK, Adler S, Baker PJ, Chen YP, Pritzl P and Couser WG, Cyclosporin A (CSA) inhibits prostaglandin E_2 (PGE_2) formation by mesangial cell (MC) in culture. *Kidney Int* **31**: 288, 1987 (abstr.).
21. Kurtz A, Pfeilschifter J, Kühn K and Koch KM, Cyclosporin A inhibits prostaglandin E_2 release from vascular smooth muscle cells. *Biochem Biophys Res Commun* **147**: 542–549, 1987.
22. Handschuhmacher RE, Harding MW, Rice J and Drugge RJ, Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* **226**: 544–546 1984.
23. Colombani PM, Robb A and Hess AD, Cyclosporin A binding to calmodulin: a possible site of action on T Lymphocytes. *Science* **228**: 337–339, 1985.
24. LeGrue SJ, Turner R, Weisbrodt N and Deman JR, Does the binding of cyclosporine to calmodulin result in immunosuppression? *Science* **234**: 68–71, 1986.
25. Berridge MJ and Irvine RF, Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature (Lond)* **312**: 315–321, 1984.
26. Pfeilschifter J, Tumour promoter 12-O-tetradecanoylphorbol 13-acetate inhibits angiotensin II-induced inositol phosphate production and cytosolic Ca^{2+} rise in rat renal mesangial cells. *FEBS Lett* **203**: 262–266, 1986.
27. Kuno M and Gardner P, Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature (Lond)* **326**: 301–304, 1987.
28. Irvine RF and Moor RM, Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external Ca^{2+}. *Biochem J* **240**: 917–920, 1986.
29. Berridge MJ, Inositol trisphosphate and diacylglycerol:

two interacting second messengers. *Ann Rev Biochem* **56**: 159–193, 1987.
30. Yamaguchi K, Hirata M and Kuriyama H, Calmodulin activates inositol 1,4,5-trisphosphate 3-kinase activity in pig aortic smooth muscle. *Biochem J* **244**: 787–791, 1987.
31. Connolly TM, Lawing WJ and Majerus PW, Protein kinase C phosphorylates human platelet inositol trisphosphate 5-phosphomonoesterase, increasing the phosphatase activity. *Cell* **46**: 951–958, 1986.