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# Cyclosporine A up-regulates angiotensin II receptors and calcium responses in human vascular smooth muscle cells

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**Cyclosporine A up-regulates angiotensin II receptors and calcium responses in human vascular smooth muscle cells.**

*Background.* The most widely used immunosuppressive drug for preventing graft rejection and treating autoimmune diseases is currently cyclosporine A (CsA). However, CsA also causes vasoconstriction, which is considered to be at the origin of CsA-induced nephrotoxicity and hypertension. To evaluate the cellular basis for these side effects, we studied the influence of CsA on the regulation of the free cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_c$) in cultured human vascular smooth muscle cells (SMCs).

*Methods.* SMCs were isolated from the medial layer of human aorta. $[Ca^{2+}]_c$ regulation was studied by fluorimetry with fura 2 and by measuring $^{45}Ca^{2+}$ effluxes. Angiotensin II (Ang II) receptors were detected by [$^{125}$I]Ang II binding.

*Results.* Pretreatment of human SMCs for 24 hours with CsA in its therapeutic concentration range (0.1 to 10.0 μM) had no effect on basal $[Ca^{2+}]_c$, but increased the $[Ca^{2+}]_c$ elevation and $^{45}Ca^{2+}$ efflux when cells were stimulated with Ang II. Half-maximal effects occurred at approximately 1 μM CsA. The CsA effects on $[Ca^{2+}]_c$ were accompanied by a nearly twofold increase in Ang II receptor number, whereas no change in affinity to Ang II was observed. CsA did not alter endothelin-1- or thapsigargin-induced $^{45}Ca^{2+}$ efflux. Increases in both $Ca^{2+}$ responses and [$^{125}$I]Ang II binding were attenuated by the transcriptional inhibitor actinomycin D. The effects of CsA did not appear to be mediated by calcineurin inhibition because cyclosporine H, which is not immunosuppressive, also increased the Ang II-induced $^{45}Ca^{2+}$ efflux.

*Conclusion.* These data suggest that CsA preferentially up-regulates the transcription of Ang II receptors, which very likely leads to vasoconstriction *in vivo* and could be at the origin of CsA-induced hypertension and nephrotoxicity in humans.

Cyclosporine A (CsA) is currently the most widely used immunosuppressive drug for preventing graft rejection and autoimmune diseases [1, 2]. However, its clinical use is limited because of its nephrotoxicity and ability to induce hypertension [3–5], and there is evidence that increased vasoconstriction is responsible for both side effects [6–9]. The renin-angiotensin system is one of the most important systems regulating blood vessel tone and is also known to play a key role in the development of some forms of arterial hypertension [10]. For example, angiotensin II (Ang II) receptor density in kidneys of spontaneously hypertensive rats is significantly higher than in control rats [11, 12]. In addition, the increased expression of the Ang II type 1A receptor ($AT_{1A}$) gene contributes to glucocorticoid-induced hypertension in rats [13]. In CsA-induced hypertension, an elevation of $AT_{1A}$ receptors in aortic smooth muscle cells (SMCs) has been demonstrated [14]. Using isolated rat aortic SMCs, we previously demonstrated that CsA augments vasoconstrictor hormone-induced elevation of free cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_c$) and $^{45}Ca^{2+}$ effluxes [15, 16].

The aims of this work were to evaluate whether CsA affects vasoconstrictor hormone-dependent $[Ca^{2+}]_c$ regulation in human cells in order to evaluate if these observations are also valid in humans, and to determine which mechanisms could be involved. In this study, we demonstrate that CsA augments Ang II-induced $Ca^{2+}$ responses and elevates Ang II receptor density in cultured SMCs isolated from human aorta. These effects do not appear to be related to the immunosuppressive activity of CsA.

## METHODS

### Chemicals and buffers

Cyclosporines A and H were gifts from Novartis Pharma (Basel, Switzerland). [Arg$^8$]vasopressin, Ang II, and endothelin-1 (ET-1) were obtained from Bachem Feinchemikalien AG (Bubendorf, Switzerland). Thapsigargin was from Sigma Chemie AG (Buchs, Switzerland). Fura 2/AM was from Molecular Probes (Eugene, OR, USA), and $^{45}Ca^{2+}$ (10 to 40 mCi/mg calcium) was

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from Amersham International (Little Chalfont, Buckinghamshire, UK). All chemicals used were of the purest grade available. Stock solutions of CsA and analogues were prepared at a concentration of $10^{-2}$ M in ethanol, and peptide hormones were in 0.1 mM acetic acid. At most, 0.1% of EtOH was present in the assays, and as tested, this did not affect the calcium response. The composition of physiological salt solution (PSS) solution was (in mM): 145 NaCl, 5 KCl, 5 HEPES, 1 $MgCl_2$, 10 glucose, pH 7.4, with either 1.2 or 0.12 mM $CaCl_2$.

### Culture of human smooth muscle cells

Smooth muscle cells were isolated from thoracic segments of human aortas from 14- to 25-year-old men six to eight hours after sudden death, which is in agreement with ethical rules as previously described [17]. For the results described here, the medial layer of a vessel from a 14-year-old boy was used for the initial culture; qualitatively similar results were also obtained with another human SMC preparation. Briefly, the vessels were opened lengthwise under aseptic conditions. Adventitia were removed mechanically. The vessels were washed with PBS, and segments were cut under sterile conditions. The media was separated mechanically from the intima along the internal elastic lamina. The pieces of media were treated with protease solution (0.225% collagenase and 0.05% elastase) for three hours at 37°C. After washing by low-speed centrifugation, the cells were seeded at a density of 3 to $5 \times 10^4$ cells/$cm^2$ into culture dishes pretreated with 0.2% gelatin. SMCs were cultured in Dulbecco's modified Eagle's medium (DMEM) with 20% of heat-inactivated human serum maintained at 37°C in an atmosphere of 95% air/5% $CO_2$, with medium changes every third day. Cells were identified by morphological evaluation as typical SMCs; 50 to 75% of them showed positive staining for smooth muscle-specific actin. The remainder were also of smooth muscle type but not sufficiently differentiated. When the cells had grown to confluence, they were trypsinized in PBS [0.05% trypsin, 0.02% ethylenediaminetetraacetic acid (EDTA)] and seeded at a density of 3 to $4 \times 10^4$ cells/$cm^2$. Cells were used from passages 7 to 11.

### Pretreatment with compounds

To study the effects of CsA or other compounds, the culture medium was removed. Cells were washed with an equal volume of DMEM without serum, and appropriate agents diluted from a $10^{-2}$ M stock solution (in ethanol) were added. In control experiments, a solution of ethanol (0.1%) in DMEM was added to cells.

### $^{45}Ca^{2+}$ efflux experiments

For $^{45}Ca^{2+}$ loading, cells were washed twice with physiological salt solution containing 0.12 mM $CaCl_2$ (PSS/0.12 mM $CaCl_2$) and were incubated in this buffer containing 1 μCi of $^{45}Ca^{2+}$ per well for 15 minutes at 37°C. Then the cells were washed rapidly four times with ice-cold PSS/1.2 mM $CaCl_2$, and $^{45}Ca^{2+}$ efflux was initiated by incubating them at 37°C in PSS/1.2 mM $CaCl_2$ (0.5 ml per well). After three, six, and nine minutes, the supernatants were removed and immediately replaced by 0.5 ml of fresh PSS/1.2 mM $CaCl_2$ at 37°C. Agonists were added in this buffer at the ninth minute. For the determination of the agonist effects, the supernatant was removed at the 11th minute. Cellular $^{45}Ca^{2+}$ content was determined by detaching the cells with 50 μl of a solution of trypsin/EDTA (0.25/1%) in PSS followed by an addition of 250 μl of a 1% sodium dodecyl sulfate (SDS) solution. The radioactivity of the supernatants and of the lysate was measured by liquid scintillation counting (Packard Tri Carb 4640).

Results are expressed as the fraction of counts per minute in the supernatant versus total cell-associated counts per minute (in cpm/min per well) as described [16].

### Measurement of $[Ca^{2+}]_c$

For $[Ca^{2+}]_c$ measurements, the cells were grown as described earlier in this article on rectangular coverslips (22.0 × 10.5 mm) pretreated with 0.2% gelatin. After reaching confluence, serum-free DMEM was added, and the cells were incubated with CsA for 20 to 24 hours. They were washed with DMEM and incubated in DMEM containing 5 μM of fura 2/AM for 30 minutes at 37°C. $[Ca^{2+}]_c$ measurement was performed on a dual-beam spectrofluorometer (Spex Fluorolog) as described [18]. The coverslip with the cells was placed at an angle of 45° in a holder inside a 1 × 1 cm quartz cuvette. Fluorescence was excited at 340 and 380 nm, and emission was measured at 510 nm. Calibrations were performed by treating the cells with ionomycin ($10^{-5}$ M) followed by 5 mM $CaCl_2$ to obtain the maximal signal and by the addition of ethylene glycol aminoethyl ether tetraacetic acid (EGTA) (10 mM) to get the minimal signal. Background fluorescence, obtained by quenching the fura 2 fluorescence with $MnCl_2$ (1 mM), was subtracted.

### [$^{125}$I]Angiotensin II binding

The cells cultured in 24-well plates were washed twice with 0.5 ml PSS/1.2 mM $CaCl_2$ (20°C), and 0.2 ml of [$^{125}$I]Ang II (1.5 to $3.0 \times 10^4$ dpm; specific activity 2200 Ci/mmol; NEN Life Science, Boston, MA, USA) in PSS containing 0.1% gelatin and 0.01% bacitracin were added together with various concentrations of unlabeled Ang II. Binding was allowed to occur during 20 minutes at 20°C. Wells were washed four times with 0.5 ml of ice-cold PSS/1.2 mM $CaCl_2$. The liquid was carefully aspirated, and 0.2 ml of 1 N NaOH was added to each well to disrupt the cells. Radioactivity of the samples was measured in a γ-counter (Gamma Master; Pharmacia,

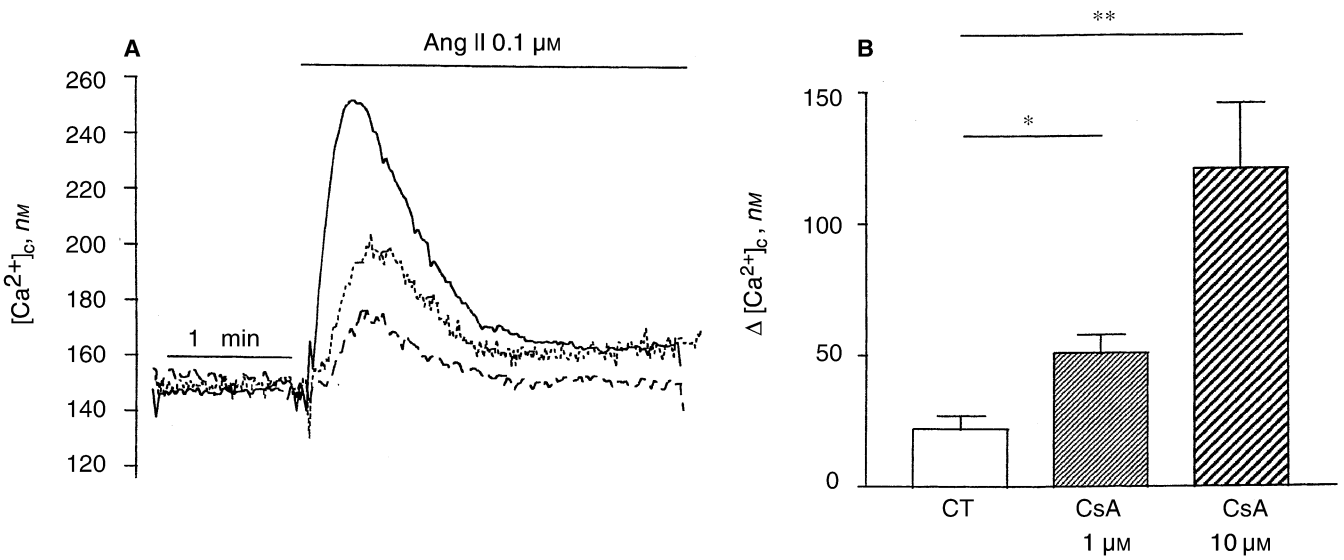


**Fig. 1. Effects of cyclosporine A (CsA) on free cytoplasmic calcium concentration ($[Ca^{2+}]_c$) (*A*) and on Ang II-induced $[Ca^{2+}]_c$ increments over basal level (*B*) in human smooth muscle cells (SMCs).** Cells were pretreated in the presence of 1 and 10 μM CsA or 0.1% ethanol (control, CT) during 24 hours. The results shown in (A) are representative of five to eight experiments. Lines are: (solid line) CsA 10 μM; (long dash) control; (short dash) CsA 1 μM; bars represent the means ± SEM. $*P < 0.05$; $**P < 0.01$.

Uppsala, Sweden). Total binding was measured with a final Ang II concentration of $1.2 \times 10^{-10}$ M and nonspecific binding with $10^{-5}$ M Ang II. To determine the binding constants ($K_D$ and $B_{max}$) of the receptors, Ang II concentration was varied between $10^{-10}$ and $10^{-8}$ M. Specific binding was calculated as the difference between total and nonspecific binding, and it amounted to approximately 20%. The amount of bound [$^{125}$I]Ang II was expressed in fmol/mg protein. Total protein was measured by the method of Bradford [19].

### Data analysis

Results are presented as the means of several independent experiments. Errors are calculated as standard errors of the mean (SEM). The unpaired Student's *t*-test was applied to evaluate significance between groups.

## RESULTS

### Elevation by cyclosporine A of angiotensin II-induced $^{45}Ca^{2+}$ efflux and $[Ca^{2+}]_c$ rise

Human smooth muscle cells (SMCs) were exposed to 1 and 10 μM CsA during 20 to 24 hours, and $[Ca^{2+}]_c$ handling was determined by fura 2 fluorimetry and by measuring $^{45}Ca^{2+}$ efflux. Basal $[Ca^{2+}]_c$ was unaltered by CsA, whereas increases in the $Ca^{2+}$ responses after stimulation with Ang II were noted (Fig. 1A). The Ang II-induced $[Ca^{2+}]_c$ increases over basal levels were 22 ± 5 nM for control cells and 51 ± 7 and 121 ± 25 nM for the cells pretreated with 1 and 10 μM CsA, respectively (Fig. 1B). After preincubation with 10 μM CsA, the difference from basal to Ang II-stimulated $^{45}Ca^{2+}$ efflux increased almost threefold (from 1.54% in control cells to 4.22% in CsA-treated cells; Table 1). Basal $^{45}Ca^{2+}$ efflux was not affected by CsA (Table 1).

To determine the sensitivity of human SMCs toward CsA, $^{45}Ca^{2+}$ efflux measurements were performed. Cells were pretreated with different CsA concentrations during 24 hours. The resulting effects on the Ang II-induced increases in $^{45}Ca^{2+}$ efflux over basal are shown in Figure 2. Already at a concentration of 0.1 μM, CsA significantly elevated the increase in $^{45}Ca^{2+}$ efflux due to Ang II. The approximate $EC_{50}$ value for the Ang II-potentiating effect of CsA was 1 μM. Basal $^{45}Ca^{2+}$ efflux was not significantly changed after preincubation with 0.1 to 10.0 μM CsA.

Angiotensin II acts on vascular SMCs via $AT_1$ receptors, which are coupled to phospholipase C [20]. $[Ca^{2+}]_c$ elevation occurs both via inositol 1,4,5-trisphosphate ($IP_3$)-induced $Ca^{2+}$ mobilization from intracellular stores and via $Ca^{2+}$ influx. CsA might elevate the Ang II responses by increasing the $Ca^{2+}$ content of intracellular pools. However, this is not likely to be the case because no changes in $^{45}Ca^{2+}$ efflux were observed after inhibition of the sarcoplasmatic $Ca^{2+}$ATPase by thapsigargin, which depletes $IP_3$-sensitive pools (Table 1). In addition, total cell accumulated $^{45}Ca^{2+}$ was not changed by CsA (not shown), confirming that CsA did not change the $Ca^{2+}$ content of intracellular pools.

The potentiating effects of CsA with respect to responses to other vasoconstrictor agonists (vasopressin and ET-1) were also investigated in these human SMCs.

**Table 1.** Effect of cyclosporine A on $^{45}Ca^{2+}$ efflux (% of total cell accumulated $^{45}Ca^{2+}$ released per minute) in the absence or presence of angiotensin II (Ang II), endothelin-1 (ET-1), [Arg$^8$]vasopressin (AVP) or thapsigargin (Tg)

| | Control | Cyclosporine A 10 μM | Significance[a] |
|---|---|---|---|
| Basal efflux | 2.95 ± 0.14 ($N$ = 23) | 3.01 ± 0.20 ($N$ = 13) | NS |
| AngII, $10^{-7}$ M | 4.49 ± 0.21 ($N$ = 26) | 7.23 ± 0.41 ($N$ = 28) | $P < 0.001$ |
| ET-1, $10^{-7}$M | 13.08 ± 0.54 ($N$ = 3) | 12.37 ± 0.40 ($N$ = 3) | NS |
| AVP, $10^{-7}$M | 3.24 ± 0.03 ($N$ = 3) | 3.11 ± 0.14 ($N$ = 3) | NS |
| Tg, $10^{-6}$M[b] | 9.69 ± 0.35 ($N$ = 2) | 9.74 ± 0.22 ($N$ = 2) | NS |

[a] Significance of the difference of Ang II-induced efflux under control conditions and after pretreatment with CsA
[b] Thapsigargin was added at 6th minute and $^{45}Ca^{2+}$ efflux was registered at 9th minute

Preliminary investigations showed that although vasopressin was devoid of a measurable effect, ET-1 strongly stimulated $^{45}Ca^{2+}$ efflux, but no potentiation by CsA was found (Table 1).

### Influence of cyclosporine A on angiotensin II receptors in human smooth muscle cells

The influence of CsA on angiotensin receptors (most likely $AT_1$ receptors) was studied by [$^{125}$I]Ang II binding to these cultured SMCs. After 24 hours of preincubation with CsA, specific binding was increased by 76 ± 25% (Fig. 3A). An analysis by Scatchard plot showed an elevation in $B_{max}$ from 11.9 ± 0.7 fmol/mg protein under control conditions to 21.1 ± 0.8 fmol/mg protein after preincubation with CsA (Fig. 3B). However, CsA did not change the affinity of $AT_1$ receptors ($K_D$ values were 1.4 ± 0.2 nM and 1.5 ± 0.1 nM without or with CsA preincubation, respectively).

### Inhibition of cyclosporine A effects on $Ca^{2+}$ fluxes and angiotensin II receptors by actinomycin D

Previously published data obtained on rat SMC [14, 21] suggest that stimulation of transcription and protein synthesis could be involved in the CsA-induced increase of the Ang II response. As shown in Figure 4, actinomycin D (1 μM) added together with CsA for 24 hours suppressed both the CsA-induced $Ca^{2+}$ potentiation and the increase in specific [$^{125}$I]Ang II binding. In cells not exposed to CsA, neither the number of Ang II receptors nor the Ang II-induced $^{45}Ca^{2+}$ efflux was affected by actinomycin D (Fig. 4).

### The cyclosporine A effect on angiotensin II-induced $^{45}Ca^{2+}$ efflux is independent of calcineurin inhibition

The effect of CsA on gene expression in T-lymphocytes is mediated by cyclophilin, which binds to calcineurin and suppresses dephosphorylation of the transcription factor NF-AT [1, 2, 22]. To evaluate whether calcineurin inhibition by the CsA-cyclophilin complex is involved in the CsA effect on Ang II-dependent calcium regulation in human SMCs, the effects of its closely related analogue cyclosporine H (CsH), which is not able to inhibit calcineurin [23], were studied. As shown in Figure 5, CsH also elevated Ang II-induced $^{45}Ca^{2+}$ efflux, although to a lesser degree. This indicates that $Ca^{2+}$ potentiation was produced independently of calcineurin inhibition.

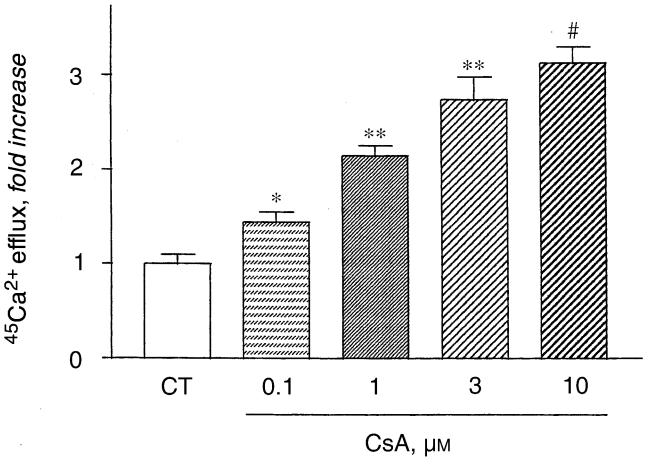


**Fig. 2. Influence of CsA on angiotensin II (Ang II)-induced increment in $^{45}Ca^{2+}$ efflux (Δ $^{45}Ca^{2+}$).** Cells were pretreated for 24 hours with the indicated concentrations of CsA. The data are normalized to Ang II-induced increment in $^{45}Ca^{2+}$ efflux over basal in cells not treated with CsA. Each point represents the mean ± SEM of three to four experiments performed in quadruplicate. *$P < 0.05$; **$P < 0.02$; #$P < 0.01$. Significance ($P$ values) was determined with respect to control (CT).

## DISCUSSION

These results show for the first time, to our knowledge, in human aortic SMCs that CsA at concentrations of 0.1 to 10.0 μM elevates Ang II-induced $[Ca^{2+}]_c$ rises and $^{45}Ca^{2+}$ efflux. The half-maximal effect occurred at approximately 1 μM, which is close to the plasma concentration of CsA in patients undergoing immunosuppressive therapy [24, 25]. In parallel, an increase in [$^{125}$I]Ang II binding, most likely to the $AT_1$ receptor, was observed. CsA increased the number of Ang II receptors 1.7-fold without changing their affinity. Both the effects on receptor numbers and on $[Ca^{2+}]_c$ regulation were suppressed by actinomycin D. These data suggest that CsA enhances

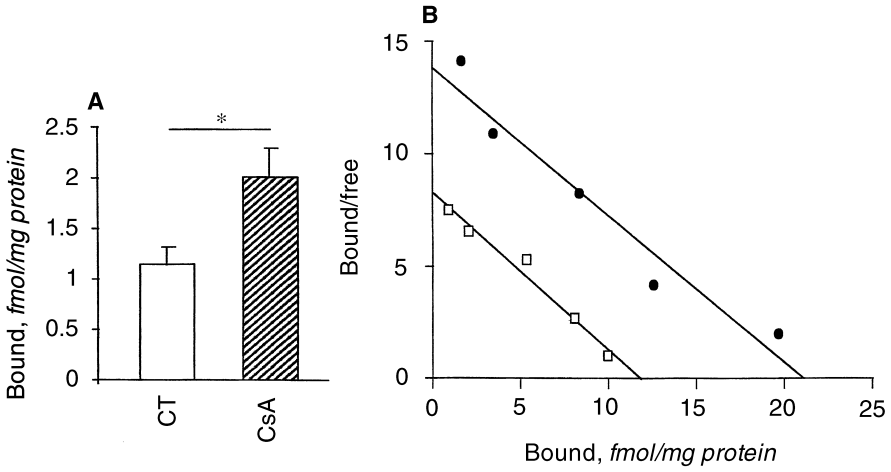


**Fig. 3. (*A*) Specific binding of [$^{125}$I]Ang II (0.12 nM) to SMCs treated with vehicle or 10 μM CsA for 24 hours (mean ± SEM of eight measurements performed in quadruplicate).** (*B*) Scatchard plot for [$^{125}$I]Ang II binding to control (CT; □) and CsA-treated (●) SMCs. Each point of the plot was obtained from three to six experiments performed in quadruplicate (*$P < 0.02$).

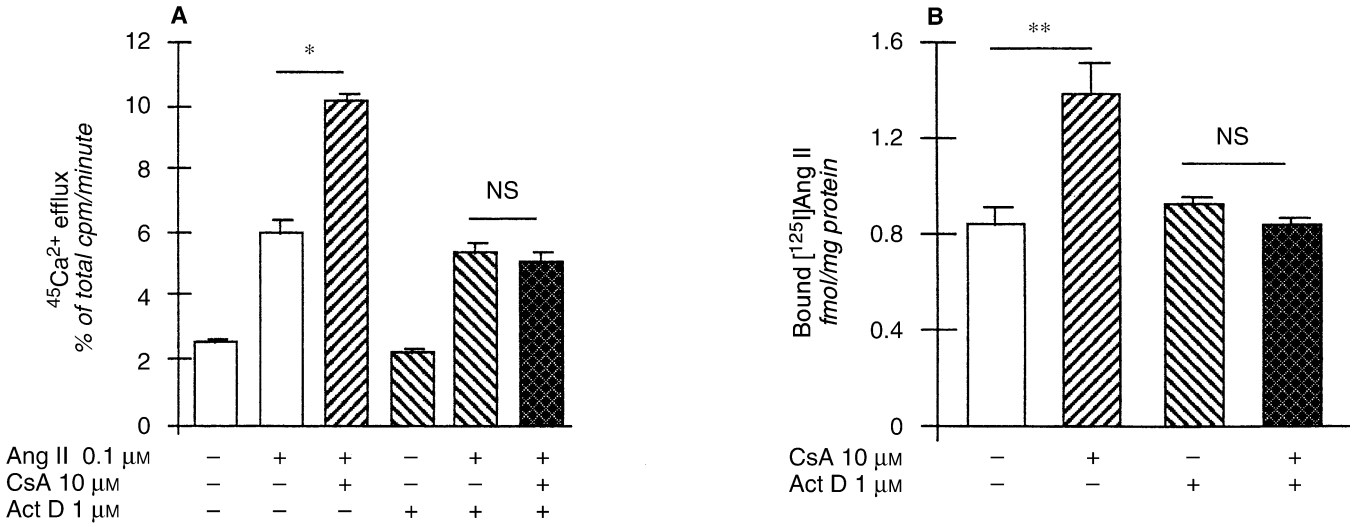


**Fig. 4. Influence of actinomycin D (1 μM) on the CsA effects on Ang II-induced $^{45}Ca^{2+}$ efflux (*A*) and [$^{125}$I]Ang II (0.12 nM) binding (*B*).** Data are mean ± SEM from three experiments performed in quadruplicate. *$P < 0.01$; **$P < 0.05$; NS, not significant.

the expression of $AT_1$ receptors in human SMCs by either stimulating $AT_1$ receptor mRNA synthesis or increasing its stability. Presumably, the rise of receptor numbers caused an increase in the $IP_3$-mediated $Ca^{2+}$ response to Ang II.

This hypothesis is supported by *in vivo* data. In rats, CsA administration caused an increase in $AT_1$ receptors in arterial walls [14]. A CsA-induced elevation of vasoconstrictor hormone receptors was demonstrated for vasopressin $V_1$ receptors in cultured SMCs from rat aorta, an effect that was accompanied by increases in $^{45}Ca^{2+}$ efflux, inositol phosphate (IP) formation, and $[Ca^{2+}]_c$ rise in response to vasopressin [21]. The responses of these rat SMCs to ET-1, serotonin, and Ang II were also increased by CsA, as well as the constriction of rat mesenteric resistance vessels [16].

These experiments show for the first time that the phenomenon of CsA-induced elevation of an agonist effect occurs in human cells. It is of interest that in human SMCs, the CsA affected only Ang II action with no change in endothelin-1–induced $^{45}Ca^{2+}$ efflux (Table 1). It is possible that CsA did not influence the expression of $ET_A$ receptors or that the effect of ET-1 on $[Ca^{2+}]_c$ was already at a maximal level and therefore could not be further potentiated.

It was demonstrated earlier that CsA is able to increase the filling of intracellular stores with releasable calcium in hepatocytes [26] and cultured rat aortic SMCs [15, 27, 28]. In our experiments, no detectable changes in intracellular $^{45}Ca^{2+}$ content and in $^{45}Ca^{2+}$ efflux in response to the SR-$Ca^{2+}$ATPase inhibitor thapsigargin were noted, indicating that the $Ca^{2+}$ pools were not affected by CsA.

What is the pathway used by CsA to increase the Ang

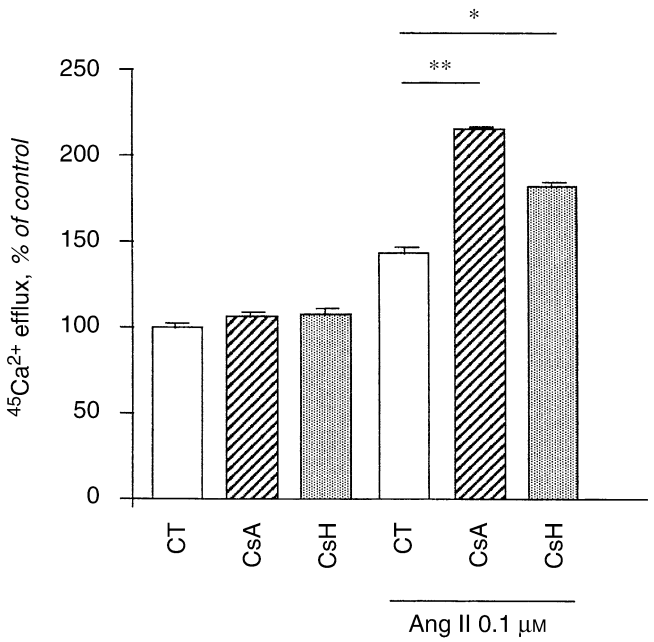


**Fig. 5. Comparison of the effects of CsA and CsH on Ang II-induced $^{45}Ca^{2+}$ efflux.** Cells were pretreated with 10 μM CsA or CsH, respectively, for 24 hours. The data are mean ± SEM from three experiments performed in quadruplicate (*$P < 0.02$; **$P < 0.01$).

II response? The well-known intracellular receptors for CsA are the cyclophilins [1, 29]. Our data indicate that the $Ca^{2+}$ potentiation occurred via a cyclophilin-independent pathway because CsH, known to have a 1000 times lower affinity than CsA to all known cyclophilins [23], was also able to increase Ang II-induced $^{45}Ca^{2+}$ efflux, although to a lesser extent. This observation is in accordance with findings on rat SMCs showing that this analogue also potentiated the $Ca^{2+}$ responses following $V_1$ receptor stimulation [16]. A dissociation between the effects of CsA on calcium responses and on immunosuppression was first shown in mesangial cells by Goldberg et al [30]. Furthermore, there appears to be a number of effects not likely to be mediated by cyclophilins, as they are produced both by CsA and CsH. It is noteworthy that the relative activities of CsA and CsH are similar to those found here. CsH displayed a fourfold lower potency as CsA in attenuating the interleukin-1β–induced increase in nitric oxide synthase expression by renal mesangial cells ($IC_{50}$ values were 3.6 and 0.9 μM, respectively) [31]. Both cyclosporines were shown to inhibit the growth of cultured keratinocytes [32, 33] and to suppress the effects of phorbol myristate acetate on mouse skin *in vivo* [34]. CsA or CsH in similar concentration ranges affected DNA and protein synthesis in the renal epithelial cell lines LLC-$PK_1$ and Madin-Darby canine kidney [35]. Thus, these data, together with our results, indicate the existence of a pathway independent of cyclophilins.

Unfortunately, there are no *in vivo* studies regarding potential nephrotoxic or hypertensive actions of CsH in humans. However, it has been shown in rats that CsH does not decrease the glomerular filtration rate after two weeks of treatment [2]. This apparent nontoxic effect may be due to the fact the CsH plasma levels in these experiments were approximately threefold lower than those of CsA [2].

CsA-induced hypertension and nephrotoxicity [3–5] are both considered to be initially due to augmentation of arteriolar contractility [6–9, 27]. Previous investigations performed on isolated arteries or on vascular SMC have demonstrated that CsA leads to an increase of the effects of $Ca^{2+}$-mobilizing hormones, resulting in an increase in peripheral resistance [15, 16, 21, 27, 28, 36].

Our data suggest that at least one of the mechanisms by which CsA enhances blood vessel contractility and blood pressure in humans could be by increasing $AT_1$ receptors and, therefore, Ang II action. This conclusion is supported by the fact that CsA-induced increase in arterial blood pressure can be attenuated by inhibitors of the renin-angiotensin system in combination with calcium antagonists [37, 38].

Similar phenomena might play a role in the development of essential hypertension. An increase in the response to $Ca^{2+}$-mobilizing agonists has been observed on cells from patients with essential hypertension [39] or on cells from spontaneously hypertensive rats [40, 41]. In addition, an increase in $AT_1$ receptor density has been observed in the peripheral tissues of spontaneously hypertensive rats [11, 12, 42] and in humans with hypertension [43]. It has been claimed that CsA-induced hypertension is similar to some forms of essential hypertension [44]. Our data about increases in Ang II-dependent $Ca^{2+}$ regulation induced by CsA in cultured human aortic SMCs, similar to those observed in essential hypertension, support such a hypothesis. Further research on the mechanisms underlying CsA-induced changes in intracellular $Ca^{2+}$ regulation could give insight into the understanding of the biochemical basis of some forms of essential hypertension in humans.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Cardenas ME, Zhu D, Heitman J: Molecular mechanisms of immunosuppression by cyclosporine, FK506, and rapamycin. *Curr Opin Nephrol Hypertens* 4:472–477, 1995
2. Borel JF, Baumann G, Chapman I, Donatsch P, Fahr A, Mueller EA, Vigouret JM: In vivo pharmacological effects of ciclosporin and some analogues. *Adv Pharmacol* 35:115–246, 1996
3. Kahan BD: Cyclosporine. *N Engl J Med* 321:1725–1738, 1989
4. Skorecki KL, Rutledge WP, Schrier RW: Acute cyclosporine nephrotoxicity: Prototype for a renal membrane signaling disorder. *Kidney Int* 42:1–10, 1992
5. Textor SC, Canzanello VJ, Taler SJ, Wilson DJ, Schwartz LL, Augustine JE, Raymer JM, Romero JC, Wiesner RH, Krom RA, Burnett JC: Cyclosporine-induced hypertension after transplantation. *Mayo Clin Proc* 69:1182–1193, 1994
6. Xue H, Bukoski RD, McCarron DA, Bennett WM: Induction of contraction in isolated rat aorta by cyclosporine. *Transplantation* 43:715–718, 1987
7. Lamb FS, Webb RC: Cyclosporine augments reactivity of isolated blood vessels. *Life Sci* 40:2571–2578, 1987
8. Kaye D, Thompson J, Jennings G, Esler M: Cyclosporine therapy after cardiac transplantation causes hypertension and renal vasoconstriction without sympathetic activation. *Circulation* 88:1101–1109, 1993
9. Roullet JB, Xue H, McCarron DA, Holcomb S, Bennett WM: Vascular mechanisms of cyclosporin-induced hypertension in the rat. *J Clin Invest* 93:2244–2250, 1994
10. Ferrario CM: Importance of the renin-angiotensin-aldosterone system (RAS) in the physiology and pathology of hypertension: An overview. *Drugs* 39(Suppl 2):1–8, 1990
11. Haws RM, Shaul PW, Arant BS Jr, Atiyeh BA, Seikaly MG: Glomerular losartan (DuP 753)-sensitive angiotensin II receptor density is increased in young spontaneously hypertensive rats. *Pediatr Res* 35:671–676, 1994
12. Wu JN, Edwards D, Berecek KH: Changes in renal angiotensin II receptors in spontaneously hypertensive rats by early treatment with the angiotensin-converting enzyme inhibitor captopril. *Hypertension* 23(6 Part 2):819–822, 1994
13. Sato A, Suzuki H, Nakazato Y, Shibata H, Inagami T, Saruta T: Increased expression of vascular angiotensin II type 1A receptor gene in glucocorticoid-induced hypertension. *J Hypertension* 12:511–516, 1994
14. Iwai J, Kanayama Y, Negoro N, Inoue T, Okamura M, Takeda T: Increased gene expression of angiotensin II type 1A receptor in aortic smooth muscle cells of cyclosporin A-induced hypertensive rats. *J Hypertens* 11(Suppl 5):S122–S123, 1993
15. Pfeilschifter J, Ruegg UT: Cyclosporin A augments angiotensin II-stimulated rise in intracellular free calcium in vascular smooth muscle cells. *Biochem J* 248:883–887, 1987
16. Lo Russo A, Passaquin AC, André P, Skutella M, Ruegg UT: Effect of cyclosporin A and analogues on cytosolic calcium and vasoconstriction: Possible lack of relationship to immunosuppressive activity. *Br J Pharmacol* 118:885–892, 1996
17. Orekhov AN, Kosykh VA, Repin VS, Smirnov VN: Cell proliferation in normal and atherosclerotic human aorta. I. Flow cytofluorometric determination of cellular deoxyribonucleic acid content. *Lab Invest* 48:395–398, 1983
18. Grynkiewicz G, Poenie M, Tsien RY: A new generation of $Ca^{2+}$ indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450, 1985
19. Bradford M: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254, 1976
20. Corriu C, Andre P, Schott C, Michel M, Stoclet JC: ANG II receptor expression and function during phenotypic modulation of rat aortic smooth muscle cells. *Am J Physiol* 266(2 Part 2):H631–H636, 1994
21. Lo Russo A, Passaquin A-C, Rüegg UT: Mechanism of enhanced vasoconstrictor hormone action in vascular smooth muscle cells by cyclosporin A. *Br J Pharmacol* 121:248–252, 1997
22. McKeon F: When worlds collide: Immunosuppressants meet protein phosphatases. *Cell* 66:823–826, 1991
23. Fliri H, Baumann G, Enz A, Kallen J, Luyten M, Mikol V, Movva R, Quesniaux V, Schreier M, Walkinshaw M, Wenger R, Zenke G, Zurini M: Cyclosporins: Structure-activity relationships. *Ann NY Acad Sci* 696:47–53, 1993
24. Sturrock ND, Lang CC, Struthers AD: Cyclosporin-induced hypertension precedes renal dysfunction and sodium retention in man. *J Hypertens* 11:1209–1216, 1993
25. Fahr A: Cyclosporin clinical pharmacokinetics. *Clin Pharmacokinet* 24:472–495, 1993
26. Nicchitta CV, Kamoun M, Williamson JR: Cyclosporine augments receptor-mediated cellular $Ca^{2+}$ fluxes in isolated hepatocytes. *J Biol Chem* 260:13613–13618, 1985
27. Rego A, Vargas R, Suarez KR, Foegh ML, Ramwell PW: Mechanism of cyclosporin potentiation of vasoconstriction of the isolated rat mesenteric arterial bed: Role of extracellular calcium. *J Pharmacol Exp Ther* 254:799–808, 1990
28. Meyer-Lehnert H, Bokemeyer D, Friedrichs U, Backer A, Kramer HJ: Cellular mechanisms of cyclosporine A-associated side-effects: Role of endothelin. *Kidney Int* 52(Suppl 61):S27–S31, 1997
29. Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW: Cyclophilin: A specific cytosolic binding protein for cyclosporin A. *Science* 226:544–547, 1984
30. Goldberg HJ, Wong PY, Cole EH, Levy GA, Skorecki KL: Dissociation between the immunosuppressive activity of cyclosporine derivatives and their effects on intracellular calcium signaling in mesangial cells. *Transplantation* 47:731–733, 1989
31. Muhl H, Kunz D, Rob P, Pfeilschifter J: Cyclosporin derivatives inhibit interleukin 1-beta induction of nitric oxide synthase in renal cells. *Eur J Pharmacol* 249:95–100, 1993
32. Ramirez-Bosca A, Kanitakis J, Haftek M, Faure M, Castells-Rodellas A, Thivolet J: Nonimmunosuppressive cyclosporin H inhibits the growth and DNA synthesis of cultured normal human epidermal keratinocytes. *Skin Pharmacol* 2:175–179, 1989
33. Amsellem C, Haftek M, Kanitakis J, Thivolet J: Effect of cyclosporins A, G, and H on normal and ichthyotic keratinocyte growth in culture. *Arch Dermatol Res* 284:173–178, 1992
34. Gschwendt M, Kittstein W, Marks F: The weak immunosuppressant cyclosporine D as well as the immunologically inactive cyclosporine H are potent inhibitors in vivo of phorbol ester TPA-induced biological effects in mouse skin and of $Ca^{2+}$/calmodulin dependent EF-2 phosphorylation in vitro. *Biochem Biophys Res Commun* 150:545–551, 1988
35. Walker RJ, Lazzaro VA, Duggin GG, Horvath JS, Tiller DJ: Structure-activity relationships of cyclosporines: Toxicity in cultured renal tubular epithelial cells. *Transplantation* 48:321–327, 1989
36. Meyer-Lehnert H, Schrier RW: Potential mechanism of cyclosporine A-induced vascular smooth muscle contraction. *Hypertension* 13:352–360, 1989
37. Hartmann A, Schweitzer G, Stratmann D, Kaltenbach M, Kober G: Effects of nitrendipine and lisinopril on blood pressure and sodium excretion in cyclosporin-associated hypertension after heart transplantation. *Cardiology* 83:141–149, 1993
38. Mimran A, Ribstein J: Angiotensin-converting enzyme inhibitors versus calcium antagonists in the progression of renal diseases. *Am J Hypertension* 7(9 Part 2):73S–81S, 1994
39. Shkhvatsabaia IK, Kravchenko AN, Avdonin PV, Men'shikov MI, Nekrasova AA: Receptor-dependent regulation of the concentration of $Ca^{2+}$ in the cytoplasm of thrombocytes in hypertensive patients. *Kardiologiia* 28:72–77, 1988

40. Orlov SN, Pokudin NI, Postnov IV: Intracellular concentration of free calcium in thrombocytes: Its characteristics in spontaneous hypertension. *Kardiologiia* 24:93–98, 1984
41. Cortes SF, Lemos VS, Corriu C, Stoclet JC: Changes in angiotensin II receptor density and calcium handling during proliferation in SHR aortic myocytes. *Am J Physiol* 271(6 Part 2):H2330–H2338, 1996
42. Song K, Kurobe Y, Kanehara H, Wada T, Inada Y, Nishikawa K, Miyazaki M: Mapping of angiotensin II receptor subtypes in peripheral tissues of spontaneously hypertensive rats by in vitro autoradiography. *Clin Exp Pharmacol Physiol* (Suppl 1):S17–S19, 1995
43. Brown L, Sernia C: Angiotensin receptors in cardiovascular diseases. *Clin Exp Pharmacol Physiol* 21:811–818, 1994
44. Luke RG: Essential hypertension: A renal disease? A review and update of the evidence. *Hypertension* 21:380–390, 1993