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# Effect of SR 33805 on arterial smooth muscle cell proliferation and neointima formation following vascular injury

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**Abstract**

The possible activity of SR 33805 ([[*N*-[dimethoxy-3,4-phenethyl]-*N*-methylamino-propoxyl]-4-benzenesulfonyl]-2-isopropyl-3-methyl-1-indole), a novel $Ca^{2+}$ channel blocker, in early atherogenesis was investigated. In vitro, SR 33805 strongly inhibited fetal calf serum-induced proliferation of cultured human aortic smooth muscle cells with an $IC_{50}$ value of $0.3 \pm 0.1$ $\mu$M ($n = 3$). In this respect, SR 33805 was several fold more active than the reference compounds: diltiazem, verapamil, nifedipine and fantofarone. SR 33805 was also a potent inhibitor of platelet-derived growth factor- or basic fibroblast growth factor-induced proliferation of human smooth muscle cells. SR33805 inhibited serum-stimulated $^{45}Ca^{2+}$ uptake in these cells, with an $IC_{50}$ value of $47 \pm 18$ nM. The effect of SR 33805 on intimal smooth muscle hyperplasia in rabbit carotid arteries subjected to air-drying endothelial injury was then investigated. After a 16-day treatment, SR 33805 (6.0 mg/kg/day p.o.) inhibited the development of intimal thickening. Under the same experimental conditions, nifedipine, verapamil, diltiazem ($2 \times 6$ mg/kg/day p.o. – 16 days) and fantofarone (12 mg/kg/day p.o. – 16 days) were inactive. These results show that SR 33805, a novel and potent $Ca^{2+}$ channel blocker, can reduce myointimal thickening following endothelial injury.

*Keywords:* $Ca^{2+}$; $Ca^{2+}$ channel antagonist; SR 33805; Smooth muscle cell; Proliferation

## 1. Introduction

The phenomenon of restenosis, which can occur following mechanical dilatation of a coronary stenosis by means of a percutaneous transluminal coronary angioplasty, needs to be studied in order to determine the clinical success of this intervention therapy in ischemic heart disease (Leimgruber et al., 1986; Shiu et al., 1985; Essed et al., 1983). In order to aid in the prevention of restenosis occurring after percutaneous transluminal coronary angioplasty, a number of drugs have been administered during or shortly after percutaneous transluminal coronary angioplasty (Thornton et al., 1984; Kent et al., 1982; White et al., 1987, 1991) but up to now, none of these compounds has reduced late-occurring restenosis arising in 30–40% of the percutaneous transluminal coronary angioplasty procedures performed in humans. The process of intimal thickening in an injured artery is the consequence of smooth muscle cell proliferation and migration from the media to the intima (Austin et al., 1985; Ross, 1986). Although the general contribution of second messengers to the development of smooth muscle cell proliferation during the formation of atherosclerotic plaques has been acknowledged for a long time, the specific role of calcium ($Ca^{2+}$) in this cellular proliferation has been suggested by work on drugs affecting $Ca^{2+}$ uptake in smooth muscle cells (Jackson et al., 1988). The close interactions between $Ca^{2+}$ and the response to vascular injury suggest that drugs which strongly inhibit $Ca^{2+}$ channel function should reduce the extent of lesion development following injury. Moreover, the actions of $Ca^{2+}$ antagonists on the progression of arterial disease have been investigated in a wide range of experimental models. In these studies (Jackson et al., 1988), a variety of dose levels and routes of administration of a range of $Ca^{2+}$ antagonists have been employed. However, the apparent disparity

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of the results obtained has prevented the emergence of a consensus on the effectiveness or otherwise of $Ca^{2+}$ channel blockers as anti-atherosclerotic or anti-restenosis agents. Nevertheless, several $Ca^{2+}$ blockers were reported to be effective in reducing myointimal proliferation after balloon injury (Jackson et al., 1988, 1989) and a significant slowing of the appearance of new coronary lesions was demonstrated by angiography in patients with mild to moderate coronary heart disease who were treated with nifedipine (Loaldi et al., 1989; Lichten et al., 1990). The mechanisms responsible for the antiatherogenic effect of $Ca^{2+}$ channel blockers are not fully understood (Jackson et al., 1988; Parmley, 1987; Weinstein, 1988). Studies performed in vitro and in vivo suggest that two main mechanisms underlie the observed effects of these drugs on atherogenesis: first, alteration of lipid metabolism in the arterial wall (Schmitz et al., 1988; Robenek and Schmitz, 1988; Stein et al., 1985; Stein and Stein, 1987) and secondly, inhibition of the proliferative activity and migration of vascular smooth muscle cells (Nilsson et al., 1985; Nomoto et al., 1988). $Ca^{2+}$ antagonists may also inhibit several platelet functions, including $Ca^{2+}$-dependent processes of adhesion and aggregation and the release of platelet factors which may participate in atherosclerosis and restenosis following injury (Ross, 1986; Fingerle et al., 1989).

The aim of this study was therefore to examine the effect of SR 33805, a novel potent $Ca^{2+}$ channel blocker (Chatelain et al., 1993), on vascular smooth muscle cell growth in vitro and to evaluate its activity on myointimal proliferation following air-drying injury of the rabbit carotid artery.

## 2. Materials and methods

### 2.1. Drugs

SR 33805 ([[*N*-[dimethoxy-3,4-phenethyl]-*N*-methyl-amino-propoxyl]-4-benzenesulfonyl]-2-isopropyl-3-methyl-1-indole) and fantofarone were from Sanofi Recherche (Brussels, Belgium). Nifedipine, verapamil and diltiazem were purchased from Sigma Chemical Co. (L'Isle d'Abeau, France). All compounds were solubilized in water and administered orally by gavage. Doses administered were determined as a function of the pharmacokinetics of the considered drugs but also with regard to their respective effect on the diastolic blood pressure in the rabbit. Basic fibroblast growth factor (bFGF) and platelet-derived growth factor (BB dimer) (PDGF-BB) were purchased from Sigma Chemical Co. (L'Isle d'Abeau, France). $^{45}Ca^{2+}$ (0.37–1.5 GBq/mg; 10–40 Ci/mg) was from Amersham (Les Ulis, France). All culture reagents were from Boehringer-Mannheim (Meylan, France).

### 2.2. In vitro experiments

#### Human and rabbit aortic smooth muscle cell growth in vitro

Smooth muscle cells were isolated from human and rabbit aortas as described previously (Paul et al., 1987). Briefly, media fragments of human aorta, withdrawn post-mortem, close to the aortic arch, or of the thoracic aorta of rabbits were incubated for 16 h at 37° C in Dulbecco's modified Eagle medium (DMEM) containing 0.15% collagenase, 5% fetal calf serum, penicillin (100 IU/ml), streptomycin (100 $\mu$g/ml) and glutamine (4 mM). After incubation, smooth muscle cells were sedimented by gentle centrifugation (400 × $g$ – 10 min), resuspended in DMEM + 10% fetal calf serum and grown at 37° C in a humidified atmosphere of 5% $CO_2$ in air. Culture medium (DMEM + 10% fetal calf serum) was changed every 3 days and a confluent smooth muscle cell monolayer was obtained after about 7 days. Cells were routinely used from the third to the sixth passage. For proliferation assays, the cells were plated sparsely ($10^3$ cells/well) in 96-well cluster plates (Nunc, Denmark) in DMEM + 0.5% fetal calf serum. After 3 days, growth-arrested cells in representative dishes were counted with a Coulter counter (Coultronics, France) and fresh medium (DMEM + fetal calf serum (5%), bFGF (10 ng/ml) or PDGF-BB (50 ng/ml)) was added to the remaining dishes. The different concentrations of the compounds to be tested were added simultaneously. For growth rate determinations, after 3 days in culture, cells were detached from triplicate wells by trypsin treatment (0.05% trypsin-0.02% EDTA) and counted in a Coulter counter. $IC_{50}$ values (concentrations which inhibit 50% of the effect) were calculated by the logistic equation fitted to the data by non-linear regression.

#### Effect of SR 33805 on $^{45}Ca^{2+}$ uptake

Calcium uptake was performed as previously described by Brown et al. (1984) with slight modifications. Cells were cultured in 24-well cluster plates (Primaria, Falcon) in DMEM containing 10% fetal calf serum. Three days before the experiment, the cells were placed in DMEM + 0.5% fetal calf serum in order to induce quiescence. Medium was removed by aspiration and replaced by 0.5 ml of physiological salt solution (PSS, composition: NaCl 145 mM, KCl 5 mM, $MgCl_2$ 1 mM, $CaCl_2$ 1 mM, glucose 5.6 mM, bovine serum albumin 1 mg/ml, Hepes/NaOH 5 mM, pH 7.4). $^{45}Ca^{2+}$ uptake was initiated by the addition of 500 $\mu$l of PSS with or without 10% fetal calf serum and $^{45}Ca^{2+}$ (10 $\mu$Ci/ml). After the indicated period of time, the $^{45}Ca^{2+}$-containing medium was removed by aspiration and the cell monolayer was washed 5 times in 30 s with ice-cold washing solution (NaCl 144 mM, $CaCl_2$ 5 mM, Tris/HCl 5 mM, pH 7.4). The cells were then digested

by incubation with 1 ml of a NaOH 0.1 N solution and cell-associated radioactivity was determined by scintillation counting. All experimental points were done in triplicate. Results were analysed by fitting a monoexponential function to the experimental data: $^{45}Ca^{2+} = {}^{45}Ca_f^{2+}$ $(1 - \exp(-\ln(2)/t_{0.5}) \times t)$ where $^{45}Ca_f^{2+}$ = maximal uptake of $^{45}Ca^{2+}$ at steady state and $t_{0.5}$ = half-life for the uptake of $^{45}Ca^{2+}$. The effect of $Ca^{2+}$ antagonists was determined as described for time-course studies except that the cells were preincubated for 1 h before the experiment with the various $Ca^{2+}$ antagonists or the vehicle. $^{45}Ca^{2+}$ uptake was determined after 15 min of incubation.

### 2.3. In vivo experiments

*Air-drying injury*

Male New Zealand rabbits (Lago, France) weighing 2.5–3 kg were used. Air-drying injury was induced by applying an air flow through the carotid artery using a modification of Fishman's method (Fishman et al., 1975; Richardson et al., 1990). The rabbits (12 animals/group) were anesthetized by i.v. injection of a mixture of acepromazine (0.3 mg/kg; Vetranquil, Sanofi-Winthrop, Gentilly, France) and ketamine (15 mg/kg; Imalgene 1000, Rhone-Merieux, Lyon, France). The left carotid artery was exposed and ligatured at two points 1.5 cm apart. A 27-gauge hypodermic needle was inserted into the proximal end of the segment. Another similar needle was used to puncture the distal end of the segment. After the lumen had been rinsed with saline, a stream of dry air was allowed to flow through the segment at 240 ml/min for 5 min. Following air-drying injury, the ligatures were removed, allowing circulation to be re-established, hemostasis was ensured and the incision was closed. The right carotid artery was manipulated but not submitted to air drying injury to serve as control.

*Tissue preparation and morphologic examination*

The various drugs were administered daily by oral route 2 days before carotid injury and daily for 2 weeks. Fourteen days after surgery, animals were anesthetized with sodium pentobarbitone (30 mg/kg i.v.). The injured carotid artery was isolated, rinsed with saline and fixed overnight with a 10% formaldehyde solution. The arterial segments were then dehydrated through graded solutions of alcohol, embedded in paraffin for serial cross-sectioning and stained with hematoxilin-eosin. Maximal plaque size was used as an indicator of smooth muscle cell proliferation. Fifty cross-sections, disposed throughout the length of the injured vessel, were examined. Morphometric analysis of arterial sections was done by use of the Biocom Imagenia 5000 image analysis system (Biocom, Lyon, France).

### 2.4. Statistical analysis of data

All data are expressed as means ± S.D. The $n$ values indicate the number of animals studied. Grouped data were analysed for significance by comparison with the vehicle-treated group using the Mann-Whitney $U$-test. The level of significance was chosen as $P < 0.05$.

## 3. Results

### 3.1. Effect of SR 33805 on rabbit and human smooth muscle cell growth in vitro

When added to growth-arrested human and rabbit smooth muscle cells, fetal calf serum, bFGF and PDGF-BB exhibited a dose-dependent mitogenic effect (Fig. 1). Human and rabbit smooth muscle cells

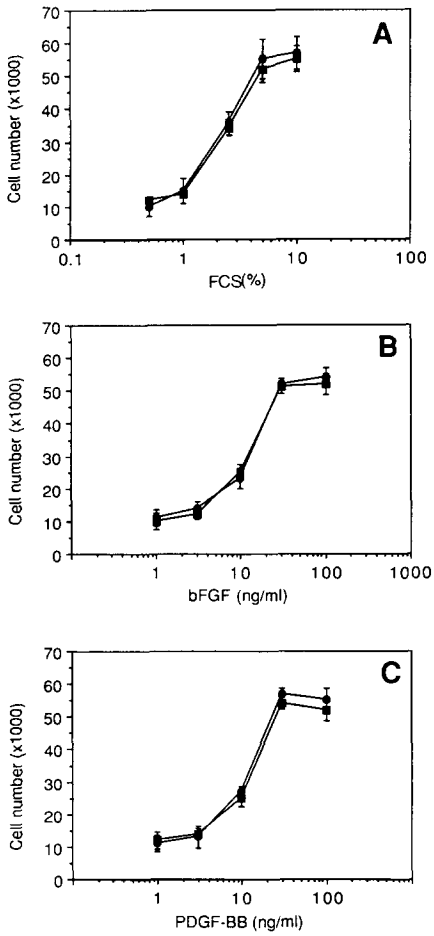


Fig. 1. Mitogemic effect of fetal calf serum, bFGF and PDGF-BB on human and rabbit smooth muscle cells. Quiescent aortic human (●) or rabbit (■) smooth muscle cells ($10^3$ cells/well) were cultured in the presence of DMEM and increasing concentrations of fetal calf serum (A), bFGF (B) or PDGF-BB (C). After 3 days in culture, the cells in triplicate wells were trypsinized and counted. Data are expressed as mean cell number ± S.D. ($n = 3$).

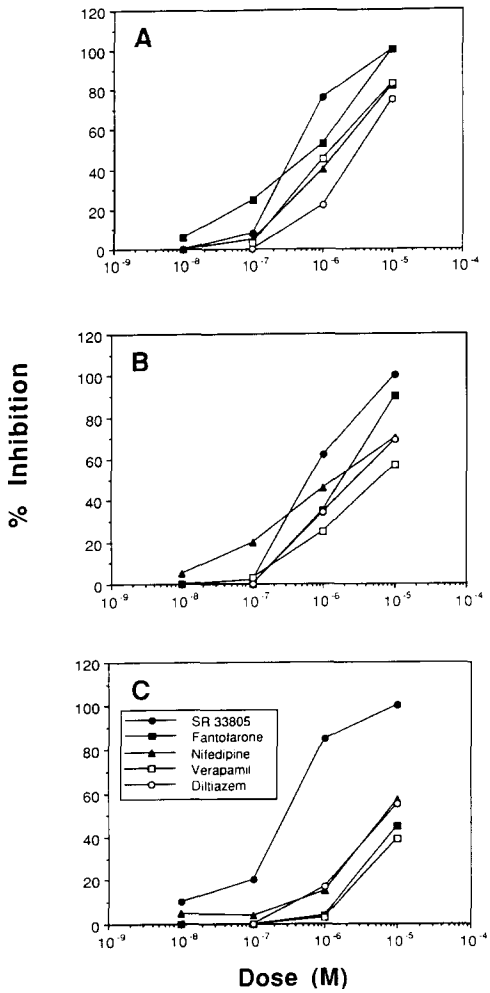


Fig. 2. Effect of SR 33805 on serum-, bFGF- or PDGF-induced human smooth muscle cell growth. Quiescent aortic human smooth muscle cells ($10^3$ cells/well) were allowed to grow in the presence of DMEM + 5% fetal calf serum (A), bFGF (10 ng/ml) (B) or PDGF-BB (50 ng/ml) (C) with increasing concentrations of SR 33805 (●), fantofarone (■), diltiazem (○), verapamil (□) or nifedipine (▲). After 3 days in culture, the cells in triplicate wells were trypsinized and counted. Data are expressed as mean percent inhibition of proliferation compared with replicate cultures grown without the inhibitor ($n = 3$).

exhibited similar sensitivities to these growth factors. As shown in Fig. 2A, SR 33805, nifedipine, diltiazem, verapamil and fantofarone inhibited in a dose-dependent manner fetal calf serum-induced proliferation of human smooth muscle cells with $IC_{50}$ values of $0.3 \pm 0.1$, $1.9 \pm 0.8$, $3.1 \pm 0.9$, $1.1 \pm 0.3$ and $0.7 \pm 0.1$ $\mu$M respectively ($n = 3$). These same compounds exhibited similar efficacy with regard to fetal calf serum-induced proliferation of rabbit smooth muscle cells (Table 1). Within the same range of concentrations, these compounds also reduced bFGF-induced smooth muscle cell growth (Fig. 2B) with $IC_{50}$ values shown in Table 1. With regard to PDGF-BB-induced proliferation of human smooth muscle cells, SR 33805 showed a potent

Table 1
Comparative effect of various $Ca^{2+}$ channel blockers on human and rabbit smooth muscle cell (SMC) growth in vitro

| Compounds | $IC_{50}$ ($\mu$M) | | | |
|---|---|---|---|---|
| | Human SMC | | | Rabbit SMC |
| | FCS | bFGF | PDGF-BB | FCS |
| SR 33805 | 0.3 ± 0.1 | 0.5 ± 0.1 | 0.2 ± 0.1 | 0.4 ± 0.1 |
| Fantofarone | 0.7 ± 0.1 | 1.7 ± 0.2 | 11.3 ± 2.9 | 10.0 ± 0.9 |
| Nifedipine | 1.9 ± 0.8 | 1.1 ± 0.6 | 6.1 ± 0.7 | 3.1 ± 1.2 |
| Verapamil | 1.1 ± 0.3 | 6.4 ± 1.8 | 20.9 ± 7.0 | 3.9 ± 0.3 |
| Diltiazem | 3.1 ± 0.9 | 2.5 ± 0.4 | 6.0 ± 1.3 | 4.5 ± 1.7 |

Values are means ± S.D. ($n = 3$).

inhibitory effect ($IC_{50} = 0.2 \pm 0.1$ $\mu$M) whereas the other reference compounds tested showed a significantly less potent inhibitory effect (Fig. 2C and Table 1). In all experiments, we did not detect any evidence of a cytotoxic effect of these compounds (tested at 10 $\mu$M) with regard to both human and rabbit smooth muscle cells as determined by trypan blue exclusion or lactate dehydrogenase release (not shown).

### 3.2. Effect of SR 33805 on serum-induced $Ca^{2+}$ uptake in human vascular smooth muscle cells

A 60-min incubation of smooth muscle cells with radiolabeled $Ca^{2+}$ resulted in a significant uptake of $^{45}Ca^{2+}$ by the cells. The total uptake of $^{45}Ca^{2+}$ reached after 60 min of incubation was not significantly different in the absence or presence of fetal calf serum. As shown in Fig. 3, fetal calf serum stimulated the rate of $^{45}Ca^{2+}$ uptake in human vascular smooth muscle cells. The half-life of accumulation was $20 \pm 2.3$ min in the absence and $8.0 \pm 2.0$ min in the presence of 5% fetal

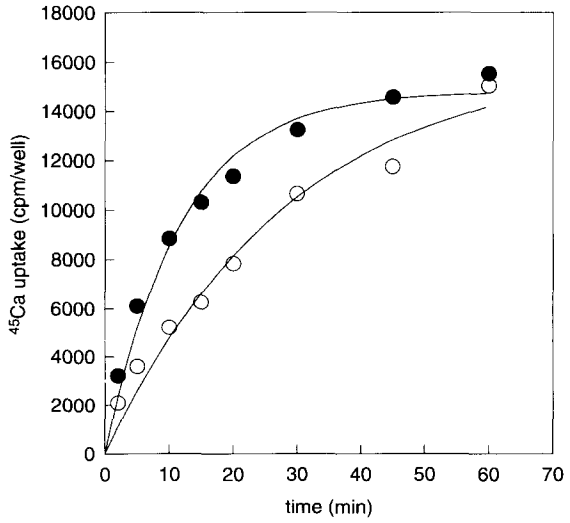


Fig. 3. Time course of $^{45}Ca^{2+}$ uptake in human vascular smooth muscle cells in the absence (○) and presence (●) of 5% fetal calf serum. Data represent the mean of three determinations.

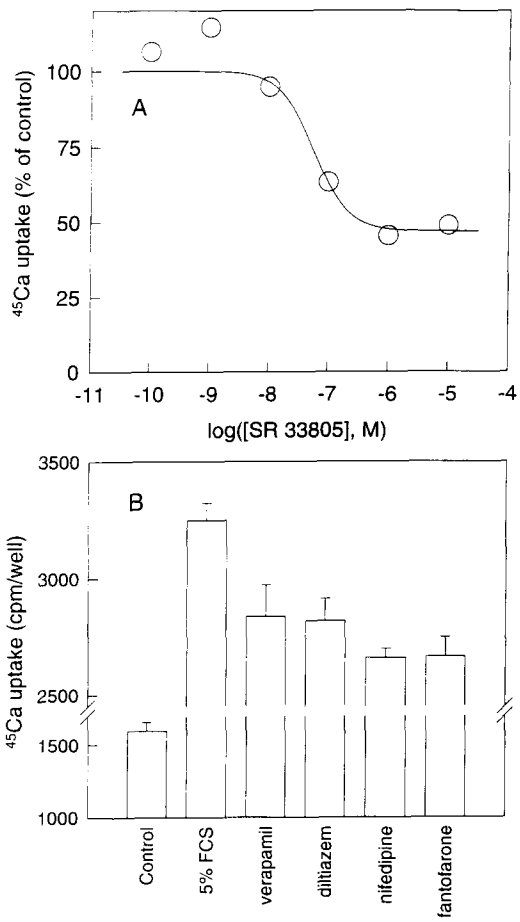


Fig. 4. Effect of $Ca^{2+}$ antagonists on serum-induced $Ca^{2+}$ uptake in human vascular smooth muscle cells. Cells were incubated for 1 h with antagonists prior to the experiments. Data are the mean of four determinations. A: Effect of increasing concentrations of SR 33805 on serum-stimulated $^{45}Ca^{2+}$ uptake. B: Effect of various $Ca^{2+}$ antagonists at a concentration of 10 $\mu$M. Values are means ± S.D. ($n = 10$).

calf serum. The effect of SR 33805, compared to other $Ca^{2+}$ antagonists, was then studied. As illustrated in Fig. 4A, SR 33805 partially inhibited serum-stimulated $^{45}Ca^{2+}$ uptake in these cells, the $IC_{50}$ value for the effect of SR 33805 in these experiments was 47 ± 18 nM. The maximal inhibition reached at high concentrations of SR 33805 represented around 50% of the serum-stimulated uptake of $^{45}Ca^{2+}$. In order to determine whether this part of the stimulated uptake was due to L-type $Ca^{2+}$ channel inhibition, other $Ca^{2+}$ channel blockers were studied. As appears in Fig. 4B, the maximal inhibition observed for chemically different $Ca^{2+}$ antagonists was identical to that obtained with SR 33805. Statistical analysis confirmed that the $^{45}Ca^{2+}$ uptake in the presence of different antagonists was identical and significantly different from the uptake in serum-free as well as in 5% fetal calf serum-containing medium.

### 3.3. Effect of SR 33805 on myointimal proliferation following air-drying injury

In the right carotid artery (that had not been subjected to air injury), there was no evidence of intimal proliferation, foam cell or platelet accumulation 14 days after the surgical procedure indicating that physical manipulation at the time of surgery but without air-drying injury was not sufficient to generate the lesion (Table 2). However, the time course of the proliferation of the intimal region of the left rabbit carotid artery that had been subjected to desiccation indicated that after denudation, smooth muscle cells were observed in the intima by day 7 (Herbert et al., 1993; Richardson et al., 1990). By 2 weeks, the intima had grown substantially and represented 42.8 ± 7.1% of the tunica media area. Medial and intimal areas were 0.4 ± 0.05 and 0.28 ± 0.07 $mm^2$ respectively. Morphometric analysis revealed that changes in the increase in the intimal surface occurred while the medial surface remained mostly unchanged (Table 2). This neointima extended to the entire circumference and approximately 80% of the length of the injured area. As determined by electron microscopy analysis, the smooth muscle cells of the neointima and the superficial media were mainly of the secretory phenotype as described by Richardson et al. (1990) (not shown).

As shown in Table 2, a 16-day oral treatment with SR 33805 (6 mg/kg/day) strongly reduced smooth muscle cell hyperplasia following de-endothelialization (54.8% inhibition – $P < 0.001$). When tested in the

Table 2
Comparative effect of SR 33805 and various $Ca^{2+}$ channel blockers on air-induced myointimal proliferation of rabbit carotid arteries

| Compounds | Dose (mg/kg/day) | Medial areas ($mm^2$) | Intimal areas ($mm^2$) | % Inhibition |
|---|---|---|---|---|
| Sham | – | 0.38 ± 0.08 | 0 | – |
| Controls | – | 0.40 ± 0.05 | 0.28 ± 0.07 | – |
| SR 33805 | 6 | 0.37 ± 0.02 | 0.12 ± 0.02 | 54.8 ± 7.4% [a] |
| Fantofarone | 12 | 0.39 ± 0.08 | 0.26 ± 0.04 | 6.7 ± 2.3% |
| Nifedipine | 2 × 6 | 0.41 ± 0.06 | 0.25 ± 0.04 | 23.2 ± 4.9% |
| Verapamil | 2 × 6 | 0.42 ± 0.05 | 0.39 ± 0.11 | −32.9 ± 7.1% |
| Diltiazem | 2 × 6 | 0.40 ± 0.02 | 0.34 ± 0.06 | −21.3 ± 3.5% |

Results shown are means ± S.D. ($n = 12$). Statistical significance (Mann-Whitney $U$-test): [a] $P < 0.001$.

same experimental conditions, within the same range of concentrations, verapamil, nifedipine, diltiazem or fantofarone did not significantly affect myointimal proliferation following air injury (Table 2).

## 4. Discussion

Restenosis following successful coronary balloon angioplasty remains one of the major unresolved problems with this procedure. Restenosis occurs in 30–40% of the dilated lesions generally 3–4 months after percutaneous transluminal coronary angioplasty (Leimgruber et al., 1986; Shiu et al., 1985; Essed et al., 1983). Although restenosis can be effectively treated by repeating the percutaneous transluminal coronary angioplasty procedure, the large number of patients requiring second and even third operations adds significantly to morbidity and cost and, despite numerous clinical trials and animal studies, no other effective non-mechanical treatment has been found to date (Herrman et al., 1993a,b).

Several mechanisms were initially proposed as possible causes of restenosis, including organized thrombus or vasospasm but a number of autopsy studies have shown that intimal hyperplasia is the main histologic finding of restenotic coronary arteries in patients with prior successful percutaneous transluminal coronary angioplasty (Bialecki et al., 1991).

During vessel damage, such as balloon injury, the release and production of growth-stimulatory and chemotactic factors occur, as do the alteration and destruction of the spatial arrangements of smooth muscle cells and the extracellular matrix. Smooth muscle cells start to proliferate as a result of exposure to growth factors and the loss of endothelial inhibitory influences. Platelet adhesion and aggregation caused by exposure of the thrombogenic subendothelium result in the release of growth factors such as PDGF, epidermal growth factor and other humoral factors such as serotonin, histamine, norepinephrine, which are mitogenic and/or chemotactic for smooth muscle cells. Other factors such as the infiltration of inflammatory cells at the injured site or shear stress might also influence the final outcome of intimal hyperplasia. Although a large number of factors may contribute to this process, $Ca^{2+}$ appears to play a fundamental role in initiating and maintaining intimal hyperplasia (Jackson et al., 1988, 1989; Loaldi et al., 1989; Lichten et al., 1990; Parmley, 1987; Weinstein, 1988). Recent studies (Bialecki et al., 1991; Gleason et al., 1991) showed that cholesterol, the level of which is increased in vascular smooth muscle cell during hypercholesterolemia, increased basal and agonist-stimulated $Ca^{2+}$ influx and $Ca^{2+}$ cytoplasmic level. Therefore, one possible method for reducing smooth muscle cell proliferation after arterial injury might be to administer drugs that inhibit $Ca^{2+}$ channel functions in vascular smooth muscle cells. Indeed, besides evidence that $Ca^{2+}$ antagonists reduce blood pressure, experimental and clinical data support the concept that they may also protect against the progression of atherosclerosis and structural changes within the vessel wall in hypertension (Loaldi et al., 1989; Lichten et al., 1990; Parmley, 1987; Weinstein, 1988). In the present study, the effects of various $Ca^{2+}$ antagonists on vascular smooth muscle cell growth in vitro and on myointimal proliferation following air-drying injury of the rabbit carotid artery in vivo, were examined and compared with those of SR 33805, a novel and potent $Ca^{2+}$ channel blocker (Chatelain et al., 1993). SR 33805 is structurally related to fantofarone but has several distinct and important differences, and recent in vivo studies have shown that SR 33805 possesses a very different profile of activity in terms of contractile activity compared to fantofarone and other $Ca^{2+}$ channel blockers (Chatelain et al., 1993). Moreover, SR 33805 showed a greater vascular selectivity than either fantofarone, nifedipine or other $Ca^{2+}$ channel antagonists (Chatelain et al., 1993).

Since the activation of $Ca^{2+}$ influx is one of the major mechanisms thought to be involved in the growth-promoting effect of various mitogens, we confirmed that the addition of fetal calf serum to quiescent smooth muscle cells induced a significant increase in the rate of $^{45}Ca^{2+}$ uptake in human smooth muscle cells. Nevertheless, the total $^{45}Ca^{2+}$ uptake was identical in the absence and presence of fetal calf serum, suggesting that fetal calf serum did not affect the size of the intracellular $Ca^{2+}$ pools, but increased the rate of exchange between the extracellular and intracellular $Ca^{2+}$ pools. An important part of the serum-stimulated $^{45}Ca^{2+}$ uptake appeared to be due to $Ca^{2+}$ entry through voltage-dependent L-type $Ca^{2+}$ channels, because $Ca^{2+}$ antagonists of different chemical structures inhibited this uptake to the same extent. Among these compounds, SR 33805 was a potent inhibitor of fetal calf serum-induced $^{45}Ca$ uptake in smooth muscle cells, giving a half-maximal effect highly similar to the $IC_{50}$ value of the compound as an inhibitor of depolarization-induced contractions in different rat arteries (Chatelain et al., 1993). This observation confirms that the effects of SR 33805 on smooth muscle cells are related to $Ca^{2+}$ channel inhibition. These data also indicate the inability of the antagonists to totally inhibit the serum-induced $^{45}Ca^{2+}$ uptake. This could be related to the activation of $Ca^{2+}$ exchange pathways unrelated to L-type $Ca^{2+}$ channels such as sodium/$Ca^{2+}$ exchange, which has been suggested to be activated in PDGF-stimulated rat vascular smooth muscle cells (Cirillo et al., 1993). Moreover, our observations confirm previous data showing that nifedipine only

partially inhibits the PDGF-induced increase of intracellular free $Ca^{2+}$ in fibroblasts (Olsen et al., 1989).

Our results show that, as well as other reference $Ca^{2+}$ antagonists, SR 33805 inhibits fetal calf serum- and bFGF-induced smooth muscle cell growth. However, with regard to PDGF-induced smooth muscle cell proliferation, SR 33805 was several fold more effective than verapamil, diltiazem, nifedipine or fantofarone. The exact mechanism whereby SR 33805 inhibits smooth muscle cell growth is not known but it seems reasonable to assume that this, as well as the other effects of SR 3380,5 is mediated by a change in $Ca^{2+}$ availability.

In this context, it is interesting to note that, due to the significant difference between SR 33805 and the other reference $Ca^{2+}$ channel blockers, the inhibitory effect of SR 33805 on PDGF-induced proliferation has a different mechanism of action from that of nifedipine, verapamil, diltiazem and fantofarone. Alternatively, it is well known that the kinetic parameters of the antagonist/$Ca^{2+}$ channel interaction and differences in membrane potential play a role in the relative potency of $Ca^{2+}$ antagonists in different tissues and explain their vascular selectivity (Lee and Tsien, 1983; Godfraind, 1992). Similar explanations may also pertain to the relative potency of compounds in a single tissue stimulated by different agonists and could account for the potency of SR 33805 as an inhibitor of PDGF-stimulated cell growth compared to the other $Ca^{2+}$ channel blockers tested. Therefore, since is now widely believed that PDGF-BB plays a pivotal role in the smooth muscle cell proliferation which occurs during plaque formation and after percutaneous transluminal coronary angioplasty, our experiments suggest that SR 33805 might be able to interfere with early steps in plaque formation. These data are particularly interesting in view of several studies showing that agents that inhibit $Ca^{2+}$ influx can also inhibit the development of atherosclerotic lesions in various animal models (Jackson et al., 1988,1989). For this reason, we determined the effect of SR 33805 on myointimal proliferation following injury of the rabbit carotid artery. In this study, SR 33805 significantly reduced the intimal hyperplasia which occurred after air-drying injury of the artery, an effect consistent with its in vitro proliferation inhibitory activity. This effect was several fold greater than that of the other $Ca^{2+}$ channel blockers tested. The mechanism by which SR 33805 reduced arterial smooth muscle cell proliferation to such an extent is unclear but data from our laboratory showed that SR 33805 possesses a high affinity ($K_d = 20$ pM) for the L-type $Ca^{2+}$ channel and demonstrates a high degree of selectivity for arterial smooth muscle over cardiac muscle compared to nifedipine, verapamil, diltiazem or fantofarone (Chatelain et al., 1993). Indeed, although its pharmacological profile appears to be that of a more vascular selective $Ca^{2+}$ channel antagonist than these compounds, there is no clear relationship between its antiproliferative and its antihypertensive properties and it is therefore unlikely that a reduction in blood pressure per se is responsible for the antiproliferative activity of SR 33805, though a reduction in carotid artery smooth muscle cell tone cannot be discounted. With regard to the mechanism of action suggested for other $Ca^{2+}$ channel blockers, several other possibilities have been discussed to explain their effect on intimal thickening following arterial injury (Henry, 1990; Schmitz et al., 1991). In addition to their inhibitory effect on smooth muscle cell proliferation (Nilsson et al., 1985; Stein et al., 1987) and migration in vitro (Nomoto et al., 1988; Nomoto et al., 1987), they interfere with extracellular matrix synthesis (Orekhov et al., 1986; Weinstein and Heider, 1987), lipid (Maggi et al., 1993) and free radical metabolism and diminish cell damage (Shridi and Robal, 1988). The resulting membrane protective effects may delay the progression of the lesions and/or reduce the response to trauma following vascular surgery.

Although the relevance of our results must await completion of appropriate clinical studies, our results suggest that SR 33805 may be useful for preventing coronary arterial diseases, including progression of atherosclerosis and restenosis after percutaneous transluminal coronary angioplasty.

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