Transforming Growth Factor-β1 Gene Activation and Growth of Smooth Muscle From Hypertensive Rats

Alex Agrotis, John Saltis, Alex Bobik

Abstract Cultured vascular smooth muscle cells derived from the spontaneously hypertensive rat (SHR) are known to replicate more rapidly than cells from the normotensive Wistar-Kyoto (WKY) rat. In this study we compared the responses of vascular smooth muscle cells from the two strains to transforming growth factor-β1 (TGF-β1) and evaluated its potential to account for the different growth properties of these cells in response to a number of vascular-derived growth factors. TGF-β1 potentiated the proliferative effects of epidermal growth factor, basic fibroblast growth factor, or the different isoforms of platelet-derived growth factor on vascular smooth muscle cells from SHR but inhibited growth factor-stimulated proliferation of vascular smooth muscle cells from WKY rats. These differential effects of TGF-β1 on proliferation could not be attributed to alterations in the expression of the type I, II, or III TGF-β receptors but appeared more related to the ability of cells to autoinduce the TGF-β1 gene. TGF-β1 caused a time-dependent increase in its own mRNA levels in vascular smooth muscle cells of WKY rats but attenuated levels in vascular smooth muscle cells of SHR. This effect was specific to TGF-β1 autoinduction since similar elevations in TGF-β1 mRNA levels were observed when vascular smooth muscle cells from the two rat strains were exposed to phorbol myristate acetate, basic fibroblast growth factor, or platelet-derived growth factor-BB. These data suggest that the production of TGF-β1 may contribute to the different growth properties of vascular smooth muscle cells from SHR and WKY rats through alterations in TGF-β1 signaling systems. (Hypertension. 1994;23:593-599.)

Key Words • transforming growth factor-β • muscle, smooth, vascular • protein-tyrosine kinase • proliferation • rats, inbred SHR.

Vascular hypertrophy is a major contributor to the increase in vascular resistance in spontaneously hypertensive rats (SHR).1 In these animals hypertrophy of the resistance vessels is, at least in part, attributed to a greater number of smooth muscle cells within the vessel wall.2 It has been suggested that differences between the ability of vascular smooth muscle cells (VSMC) from SHR and normotensive Wistar-Kyoto (WKY) rats to respond to the mitogenic influence of vascular-derived growth factors could account for this “proliferative hypertrophy” in genetic hypertension.3•6 One of these vascular-derived growth factors, transforming growth factor-β1 (TGF-β1), is a multifunctional protein that regulates the growth and differentiation of a wide variety of cells in culture.7•8 In VSMC TGF-β1 can induce both cellular hypertrophy and polyplody as well as stimulate and/or inhibit proliferation.9•10 For example, TGF-β1 has been shown to stimulate the proliferation of both sparse and dense cultures of human smooth muscle cells.11 Also, TGF-β1 inhibits serum-stimulated proliferation of rat VSMC, seeded at low densities; at high densities the opposite effect on proliferation is observed.10•12 These opposing effects of TGF-β1 are elicited after its interaction with multiple receptors on the surface of smooth muscle cells.7

Several lines of evidence suggest that TGF-β1 may play an important role in the development of hypertension. Initially, Sarzani et al14 demonstrated elevated TGF-β1 mRNA expression in the aorta of rats whose hypertension was induced with deoxycorticosterone and salt. Others have shown increased expression of TGF-β1 mRNA levels in the aorta of SHR and WKY rats as their age increases from 5 to 40 weeks.15 Elevated mRNA levels encoding TGF-β1 have also been reported in VSMC from SHR, compared with those from WKY rats, cultured in the presence of serum.16 More recently it has been suggested that increased TGF-β1 expression and activation is associated with augmented proliferation of VSMC from SHR.17 We have previously shown that TGF-β1 interacts with tyrosine kinase–activating growth factors such as basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), or epidermal growth factor (EGF) to potentiate their mitogenic activity in VSMC from the SHR.18

The aim of the present study was twofold: (1) to investigate whether the ability of TGF-β1 to potentiate the effects of tyrosine kinase–activating growth factors was unique to VSMC from SHR and (2) to examine the relation between TGF-β1 and growth factor–stimulated proliferation of VSMC from SHR and WKY rats. Our results indicate that TGF-β1 inhibits growth factor–stimulated proliferation of VSMC from normotensive WKY rats, whereas under identical conditions there is potentiation of proliferation in VSMC from SHR. These differential effects appear to be due to alterations in intracellular signaling mechanisms stimulated by TGF-β1 and involved in the regulation of proliferation. In addition, the production of endogenous TGF-β1 by

Received May 13, 1993; accepted in revised form February 9, 1994.

From the Baker Medical Research Institute and Alfred Hospital, Prahran, Victoria, Australia.

Correspondence to Dr Alex Agrotis, Baker Medical Research Institute, Commercial Rd, Prahran, 3181 Victoria, Australia.
cells after stimulation with growth factors may account for the well-known differences in the growth properties of VSMC from the two rat strains.

**Methods**

**Animals**

Male SHR and WKY rats (weight, 250 to 300 g) were bred at the Baker Medical Research Institute from stock originally supplied by Y. Yamori. Immediately before excising the aorta, for dispersion into single cells (see “Methods”), the animals were deeply anesthetized with halothane. This procedure was approved by the Baker Institute–Alfred Hospital Animals Experimentation Committee and conformed to the guidelines of the Australian National Health and Medical Research Council.

**Materials**

Fetal calf serum (FCS), penicillin G, and Dulbecco’s phosphate-buffered saline (PBS) were purchased from Commonwealth Serum Laboratories. We obtained Dulbecco’s modified Eagle’s medium (DMEM) from FLOW Laboratories. Tissue culture dishes were purchased from Sterilin Ltd. Collagenase type 1 (C-0130), elastase (E-0258), EGF, phenylmethylsulfonyl fluoride (PMSF), and leupeptin were obtained from Sigma Chemical Co. Porcine transforming growth factor-β1 was obtained from British Bio-Technology Ltd. Human [51]TG-β1 was purchased from Dupont Australia Ltd. bFGF and methyl-[3H]thymidine were obtained from Amersham. PDGF-AA and PDGF-AB, bovine serum albumin (BSA), and the random priming DNA labeling kit were obtained from Boehringer-Mannheim. PDGF-BB was obtained from Zymogenetics. A rat TG-FG-β1 cDNA probe consisting of a 1-kb fragment spanning the major coding region of the TG-β1 precursor (Qian et al) was provided by Dr A. Roberts, Laboratory of Chemoprevention, National Institutes of Health, Bethesda, Md. A 30-base oligonucleotide that hybridizes to the 18s rRNA species was provided by Dr Z. Krozowski, Molecular Hypertension Laboratory, Baker Medical Research Institute, Melbourne, Australia.

**Isolation and Culture of Aortic Smooth Muscle Cells**

Primary cultures of VSMC were prepared by enzyme dispersion of aortic media from 12- to 14-week-old SHR and WKY rats as previously described.6 Examination of the cultures by phase-contrast microscopy indicated a “hills and valleys” pattern at confluency, a well-known characteristic of VSMC in culture. The identity of smooth muscle cells in our cultures was verified by immunochemical analysis using antibodies for smooth muscle myosin.20 These “primary” smooth muscle cells were subsequently passaged every week and grown in 10% FCS/DMEM using 90-mm tissue culture dishes. Cells between primary and fourth passages were used in all experiments.

[3H]Thymidine Incorporation and Cell Proliferation

Cells were grown to confluence (1 to 2×10^4 cells) in 24-well tissue culture dishes. At this time the medium was replaced with 1.0 mL of DMEM, and cells were incubated for an additional 48 to 72 hours. After 24 hours of serum deprivation, in excess of 85% of the VSMC were present in G0/G1; assessed with a fluorescent-activated cell sorter; the remainder were present in the G2/M phase.21 Growth factors, EGF (50 ng/mL), and/or TG-FG-β1 (1 ng/mL) (dissolved in DMEM containing 1% BSA) were then added to the quiescent cells, in a total volume of 0.5 mL of DMEM alone. At the indicated times the medium was aspirated, and the cells were washed once with 1.0 mL of DMEM and then incubated for an additional 2 hours in 1.0 mL DMEM containing methyl-[3H]thymidine (1 μCi/mL). At the end of this period, the medium was removed and cells washed three times with 1.0 mL of ice-cold Dulbecco’s PBS before the addition of 0.5 mL of ice-cold 10% trichloroacetic acid for 15 to 30 minutes. After an additional wash with 10% trichloroacetic acid, the cells were solubilized in 1 mol/L NaOH, neutralized with 1 mol/L HCl, and radioactivity was then determined by scintillation spectrometry.

In the proliferation studies VSMC (1×10^4) were plated into 24-well tissue culture dishes in 1.0 mL of 10% FCS/DMEM. The next day the medium was replaced with 1.0 mL of DMEM, and cells were cultured for an additional 24 hours. Growth factors were then added (TG-FG-β1 [1 ng/mL], EGF [50 ng/mL], bFGF [25 ng/mL], PDGF-AA [400 ng/mL], PDGF-AB [200 ng/mL], and PDGF-BB [200 ng/mL]) in 0.5 mL of DMEM containing insulin and transferrin (4% Monomed A, Commonwealth Serum Laboratories). The growth factor-containing medium was replaced every 2 days, and cell number was then determined at indicated times using a Coulter counter. We have previously demonstrated that the aforementioned concentrations of growth factors induce maximal incorporation of [3H]thymidine into VSMC of SHR and WKY rats (Saltis et al).22

**Affinity Labeling of Transforming Growth Factor-β Receptors**

The identification of TG-FG-β receptors by affinity labeling was performed essentially as described by Massagué.23 Cells were grown in DMEM containing 10% FCS to confluency (≈2×10^4 cells) in 12-well tissue culture dishes. They were washed with 2.0 mL of binding buffer per well (DMEM, 1% BSA, 20 mM/L HEPES [pH 7.4]) and then incubated in 2.0 mL of this medium for 2 hours at 37°C. After additional washing of the cells, they were incubated for 3 to 4 hours at 4°C in 0.5 mL binding buffer containing 150 mmol/L [3H]TGFG-β1, either alone or in the presence of a 100-fold excess of unlabelled TG-FG-β1. The cells were then washed three times with 2.0 mL binding buffer, followed by an additional wash with 2 mL of PBS buffer (without BSA). Disuccinimidyl suberate, freshly dissolved in dimethyl sulphoxide, was added to the cells in 0.5 mL PBS to a final concentration of 0.5 mmol/L. After 15 minutes at 4°C on an orbital shaker, the cells were washed twice with 2.0 mL PBS containing 1 mmol/L PMSF and 10 μg/mL leupeptin, scraped from the plastic surface, and collected by centrifugation at 10,000×g for 10 minutes at 4°C. Sodium dodecyl sulfate (SDS)—polyacrylamide gel electrophoresis sample buffer (50 to 100 μL) was added to the pellets, and samples were boiled for 5 minutes, followed by centrifugation at 10,000×g for 10 minutes at 4°C. The supernatant solutions were stored at −20°C for 1 to 2 days before electrophoresis was performed on a 6.5% resolving gel under nonreducing conditions. Solubilized smooth muscle cells, adjusted to the same cell number, were subjected to electrophoresis. After electrophoresis, gels were fixed in 25% trichloroacetic acid and stained with 0.1% Coomassie brilliant blue. Autoradiography of dried gels was performed with Kodak X-Omat AR film using Dupont Lightning Plus screens.

**RNA Isolation and Analysis**

Total RNA was extracted from confluent VSMC cultures (60-mm tissue dishes) by the guanidinium thiocyanate method of Chomczynski and Sacchi24 and then electrophoresed (5 to 15 μg) on 1% agarose/(2.2 mol/L) formaldehyde gels. After electrophoresis, the gels were treated with 50 mmol/L NaOH for 20 minutes, neutralized with a 0.5 mol/L Tris solution (pH 7.4) containing 1.5 mol/L NaCl for 20 minutes, and then equilibrated with 20× SSC for 20 to 40 minutes (20× SSC=3 mol/L NaCl and 0.3 mol/L Na citrate [pH 7.0]). RNA was transferred to 0.45-μm Biortrans Membranes (ICN), UV fixed for 5 minutes, and then baked at 80°C for 1 to 2 hours. Prehybridization was carried out at 65°C for 1 to 4 hours in a
Potentiated maximal rates of EGF-stimulated [3H]thy-
factors such as EGF. In VSMC from SHR, TGF-β1 
amerately 25% (Fig 1).
ated by TGF-β1 varied, ranging from 30% to 100% (Fig
increased, [3H]thymidine incorporation into VSMC from 
same conditions TGF-β1 attenuated, rather than in-
oration into VSMC from SHR and WKY rats. When 
position was approximately 200% (Fig 1). Under the 
concentration the increase in [3H]thymidine incor-
ferences. The enhancing effect of TGF-β1 on EGF-stim-
ulated [3H]thymidine incorporation was observed at 
concentrations approximately 10-fold lower than its 
haracteristics of growth factor–induced mitogenesis in 
WI cultures and increased in SHR cultures (Fig 4). In 
and second round of washes were performed with 
phosphate buffer containing 1% SDS, 40 mmol/L NaHPO4,
and 1 mmol/L EDTA before being sealed in plastic and
posed to Kodak X-Omat AR film with intensifying screens 
for 2 days at -70°C. The resulting autoradiographs were 
analyzed by laser densitometry at 600 nm.

Statistical Analysis

The significance of differences within and between strains 
was assessed by one-way ANOVA. Results have been ex-
pressed as mean ± SEM of three to seven experiments.

Results

Transforming Growth Factor-β1 and 
[3H]Thymidine Incorporation in DNA

Initially we compared the effects of increasing concen-
trations of TGF-β1 alone on [3H] thymidine incorpora-
tion into VSMC from SHR and WKY rats. When con-
fuent and serum-deprived VSMC from SHR were 
exposed to TGF-β1 for 24 hours, they increased their 
incorporation of [3H] thymidine into DNA. This stimu-
latory effect of TGF-β1 became apparent at concentra-
tions above 0.05 ng/mL and was maximal at 1 ng/mL; at 
this concentration the increase in [3H] thymidine incor-
poration was approximately 200% (Fig 1). Under the 
same conditions TGF-β1 attenuated, rather than in-
creased, [3H] thymidine incorporation into VSMC from 
WKY rats. At 1 ng/mL of TGF-β1, [3H] thymidine incor-
poration into these cells was reduced by approximately 
25% (Fig 1).

These differential effects of TGF-β1 on [3H] thymi-
dine incorporation into VSMC from SHR and WKY 
rats were also observed in the presence of growth 
factors such as EGF. In VSMC from SHR, TGF-β1 
potentiated maximal rates of EGF-stimulated [3H] thy-
midine incorporation; the extent of potentiation medi-
at ed by TGF-β1 varied, ranging from 30% to 100% (Fig
2B and Reference 18). The enhancing effect of TGF-β1 
on growth factor–stimulated DNA synthesis in VSMC 
from the SHR was evident over the concentration range 
0.1 to 1.0 ng/mL (Fig 2D), similar to that seen on 
[3H] thymidine incorporation in serum-deprived cells 
(Fig 1). This action of TGF-β1 on [3H] thymidine incor-
poration became evident approximately 18 hours after 
exposing the cells to the two growth factors and per-
sisted for the ensuing 30 hours (Fig 2B). In contrast to 
these results, TGF-β1 inhibited by 10% to 50% EGF-
stimulated DNA synthesis in VSMC from WKY rats 
(Fig 2A). This effect of TGF-β1 was not simply a conse-
quency of any prolongation of the GI phase but rather 
appeared to be the consequence of a smaller number 
of cells being stimulated by EGF to enter the S 
phase. This inhibitory effect of TGF-β1 on EGF-stimu-
lated [3H] thymidine incorporation was seen at concen-
trations approximately 10-fold lower than its enhanc-
ing effects on growth factor–induced mitogenesis in 
VSMC from SHR (Fig 2C). Even when TGF-β1 con-
centrations were increased in VSMC from WKY rats 
to those that caused maximum enhancement of 
EGF-induced DNA synthesis in VSMC from SHR, 
there was still no enhancement of mitogenesis.

Transforming Growth Factor-β1 
and VSMC Proliferation

The effects of TGF-β1 on the proliferation of sparse 
cultures of VSMC from SHR and WKY rats exposed to 
EGF, bFGF, or the isoforms of PDGF (PDGF-AA, 
-AB, -BB) are shown in Fig 3. In VSMC from WKY rats, 
TGF-β1 inhibited by 70% to 95% mitogen-stimulated 
proliferation (Fig 3A). This inhibition by TGF-β1 was 
evident at concentrations that inhibited the incorpora-
tion of [3H] thymidine into these cells (Fig 2). In 
contrast, TGF-β1 enhanced growth factor–stimulated 
proliferation of VSMC from SHR (Fig 3B). The mag-
itude of this stimulation varied with the different 
growth factors and ranged from 12% to 90%. No 
inhibition of proliferation by TGF-β1 was observed in 
VSMC from SHR.

We have previously demonstrated that different 
mechanisms appear to be responsible for the regula-
tion of growth rates of VSMC and the density at which 
these 
cells became refractory to growth factor stimulii. 
Hence, it was also of interest to examine whether 
TGF-β1 differentially affected the density at which 
quiescence was attained by growth factor–stimulated 
VSMC from SHR and WKY rats. When proliferating 
VSMC were exposed to high concentrations (1 ng/mL) 
of TGF-β1, the density at which VSMC became refrac-\ntory to the proliferative effects of EGF was lowered in 
WKY cultures and increased in SHR cultures (Fig 4). In 
VSMC from WKY rats this reduction in cell density was 
approximately 60% (P<.01), whereas in the cultures 
from the genetically hypertensive rats the increase was 
greater than 200% (P<.01) (Fig 4).

Transforming Growth Factor-β1 Gene Expression

Because the effects of TGF-β1 on proliferation and 
the density at which proliferating VSMC from SHR and 
WKY rats became refractory to growth factor stimuli 
differed markedly, it was also of interest to examine 
whether other TGF-β1 responses might also be differ-
entially affected. Thus, we compared the relative abili-
ties of TGF-\(\beta\)1 to activate TGF-\(\beta\)1 gene transcription in VSMC from the two strains. Previously, Van Obbergen-Schilling and coworkers\(^2\) demonstrated that TGF-\(\beta\)1 induces its own mRNA levels in a number of cell lines.

When quiescent VSMC from WKY rats were exposed to TGF-\(\beta\)1, there was a time-dependent increase in TGF-\(\beta\)1 mRNA levels (major transcript, 2.5 kb; minor transcript, 1.9 kb); maximal induction (twofold to threefold) occurred after 24 hours (Fig 5A). In contrast, when VSMC from SHR were exposed to TGF-\(\beta\)1, there was an approximately 20% to 50% reduction in mRNA levels for TGF-\(\beta\)1 after 24 hours compared with levels in quiescent cells. To examine whether this differential expression of TGF-\(\beta\)1 mRNA between the strains was due to alterations in the promoter region of the TGF-\(\beta\)1 gene, quiescent VSMC were also exposed to phorbol myristate acetate (PMA). This phorbol ester has previously been shown to stimulate TGF-\(\beta\)1 gene expression in a manner similar to that of TGF-\(\beta\)1 via discrete promoter DNA sequences that bind the transcription factors AP-1 and Sp1.\(^2\) Similar levels of TGF-\(\beta\)1 mRNA were attained in VSMC from SHR and WKY rats after 16 hours of exposure to this activator of protein kinase C (Fig 5B).

Furthermore, when VSMC from SHR were exposed to either bFGF or PDGF-BB, the mRNA levels for TGF-\(\beta\)1 were elevated to the same extent as observed in VSMC from WKY rats (Fig 6). Increases in TGF-\(\beta\)1 mRNA were already apparent 6 hours after exposure to these growth factors; maximal induction occurred between 6 and 16 hours.

Receptor Subtypes for Transforming Growth Factor-\(\beta\)1

Although we have previously shown that the total number of receptor sites for TGF-\(\beta\)1 does not differ on VSMC from SHR and WKY rats,\(^2\) differences in the expression of receptor subtypes could be responsible for its differential affects on VSMC growth, as has been reported by Boyd and Massague\(^3\) in mink lung epithelial cells; it could also contribute to differences in TGF-\(\beta\)1 autoinduction. When \([\text{125I}]\)TGF-\(\beta\)1, bound to serum-deprived VSMC from SHR and WKY rats, was covalently cross-linked with disuccinimidyl suberate and the ligand-receptor complexes subjected to SDS-polyacrylamide gel electrophoresis and autoradiography, three major bands were detected: Mr = 53 kDa, 75 kDa, and 300 kDa, corresponding to type I, type II, and type III receptor subtypes, respectively (Fig 7). At the concentrations of \([\text{125I}]\)TGF-\(\beta\)1 used in the binding studies (150 pmol/L), cross-linking was greatest to the high-molecular-weight type III proteoglycan TGF-\(\beta\) binding protein and least to the type II TGF-\(\beta\) receptor. There was no apparent difference in the electrophoretic mobilities of the three TGF-\(\beta\) receptor subtypes.

Discussion

This study demonstrates that TGF-\(\beta\)1 differentially regulates growth factor–stimulated proliferation of VSMC from SHR and WKY rats. Both the rate of proliferation and cell density at which quiescence is achieved by VSMC from WKY rats are reduced by the presence of TGF-\(\beta\)1, whereas in VSMC cultures from SHR enhancement in both growth parameters occurs.
These differential effects of TGF-β1 on VSMC from the two strains are not apparently a consequence of differences in TGF-β receptor subtype expression; rather, it would appear that an alteration in intracellular signaling mechanisms could account for these opposing actions of TGF-β1. These observations, together with our finding that tyrosine kinase-activating growth factors are equipotent in their ability to elevate TGF-β1 mRNA levels, suggest that the observed differences in the growth properties of VSMC from SHR and WKY rats may be mediated through the production of endogenous TGF-β1.

Despite intensive investigation, the mechanisms by which TGF-β1 elicits its diverse array of biological responses remain unknown.7,8 The effects of TGF-β1 appear to be mediated by three cell surface receptors termed types I, II, and III with apparent molecular weights of 55 kD, 75 kD, and 280 kD, respectively.31 Selective loss of the type I and/or type II TGF-β receptors has been associated with the inability of TGF-β1 to inhibit the proliferation of some cell types.32 Such a mechanism does not account for the lack of TGF-β1 action to inhibit growth factor-stimulated proliferation of VSMC from SHR. We have previously demonstrated that VSMC from SHR and WKY rats possess similar numbers of high-affinity TGF-β receptors.6 They also express all three TGF-β receptor subtypes in roughly similar proportions, as determined by affinity cross-linking studies. Most probably alterations in events distal to TGF-β binding to its receptors account for the differential ability of this growth factor to affect the proliferation of VSMC from SHR and WKY rats. It is possible that key intracellular proteins, such as the retinoblastoma gene product33 or the p34cdc2 kinase,34 thought to be involved in TGF-β-mediated inhibition of proliferation, may differ in VSMC from SHR and WKY rats. Recently it has been demonstrated that the concentration of G proteins, in particular some of the Gβ subtypes, is increased in VSMC from SHR;35 enhanced Gβ has also been associated with increased [3H]thymidine incorporation stimulated by TGF-β1.36 Whether one or more of these processes account for the observed differences in TGF-β1 responses we observed requires further study.

A general response of many cells in culture after stimulation with TGF-β1 is an increase in TGF-β1 mRNA expression.27 Similarly, in VSMC from WKY rats TGF-β1 positively regulates its own expression. However, TGF-β1 is ineffective in activating its own gene in VSMC from the SHR. This inability of VSMC from SHR to elevate TGF-β1 mRNA levels is specific for TGF-β1 because other growth factors, such as PDGF-BB, bFGF, and PMA, all increase TGF-β1 mRNA levels to a similar extent in VSMC from SHR and WKY rats. Because both PMA and TGF-β1 elevate TGF-β1 mRNA levels by stimulating gene expression through the AP-1 binding site in the TGF-β1 gene promoter region,28 it would appear that signals before stimulation...
through the AP-1 site are responsible for the impaired ability of TGF-β1 to elevate TGF-α1 mRNA in VSMC from SHR. Whether the same intracellular mechanisms also contribute to the differential ability of TGF-β1 to modulate cell proliferation in VSMC of SHR and WKY rats remains to be determined.

Exaggerated expression of TGF-β1 mRNA has recently been associated with enhanced growth of serum-stimulated VSMC from SHR.16 Our observations suggest that the enhanced proliferative ability of these cells is more likely to be the consequence of the enhanced responsiveness of the cells to mitogenic stimulation in the presence of TGF-β1. Stimulation of VSMC from SHR and WKY rats to enter the mitotic cell cycle by PDGF-BB or bFGF was associated with similar increases in TGF-β1 mRNA levels. Despite the essentially similar elevations in TGF-β1 mRNA levels in these circumstances, enhanced replication of VSMC from SHR compared with those of WKY rats was still observed in the presence of these growth factors. Our observations raise the possibility that endogenously biosynthesized and activated TGF-β1 may be responsible for the different growth properties of VSMC from the two rat strains. Replicating VSMC are known to secrete a variety of proteases, including plasminogen activator37 and collagenase.38 Furthermore, in serum-containing medium both proteinase inhibitors and an antisense oligonucleotide to TGF-β1 have recently been shown to attenuate DNA synthesis in VSMC from SHR.17 In this instance serum-derived plasmin may have also contributed to the activation of latent TGF-β1.39 In the future it will be of interest to determine whether a significant proportion of the latent TGF-β1 that accumulates when VSMC are cultured in serum-free medium is activated during exposure to growth factors.

In summary, our findings indicate that TGF-β1 differentially affects the proliferation of vascular smooth muscle from SHR and WKY rats. Our observation that TGF-β1 mRNA levels are elevated to a similar extent

![Fig 5. A, Blot shows transforming growth factor-β1 (TGF-β1) mRNA expression in vascular smooth muscle cells (VSMC) from spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Total RNA was isolated from control, serum-deprived cells from WKY rats and SHR as well as from VSMC incubated with TGF-β1 (1 ng/mL) for periods of 2 to 24 hours. Northern blot analysis was performed using a specific 32P-labeled cDNA probe for TGF-β1, as described in 'Methods.' W and S indicate VSMC from WKY and SHR rats, respectively. B, Blot shows TGF-β1 mRNA levels in VSMC after exposure to phorbol myristate acetate (PMA). TGF-β1 mRNA levels were assessed after exposing VSMC of WKY (W) rats and SHR (S) to 1 μmol/L PMA for 16 hours. Panels beneath autoradiographs in both A and B represent hybridization to an 18s rRNA probe, indicating similar RNA loading.](http://hyper.ahajournals.org/)

![Fig 6. Blots show growth factor-stimulated transforming growth factor-β1 (TGF-β1) mRNA expression in vascular smooth muscle cells. Control and quiescent cells (Q) (∼×10⁶) from Wistar-Kyoto (WKY) rats or spontaneously hypertensive rats (SHR) were incubated with either (A) basic fibroblast growth factor (bFGF; 25 ng/mL) or (B) platelet-derived growth factor (PDGF)-BB (200 ng/mL) for the indicated times in hours; total RNA was then extracted and Northern blot analysis performed using a specific 32P-labeled cDNA probe for TGF-β1. Panels beneath autoradiographs in both A and B represent hybridization to an 18s rRNA probe, indicating similar RNA loading.](http://hyper.ahajournals.org/)

![Fig 7. Autoradiograph shows transforming growth factor-β (TGF-β) receptor expression on vascular smooth muscle cells. TGF-β receptor subtypes on vascular smooth muscle cells were estimated after 125I TGF-β1 binding and cross-linking with disuccinimidyl suberate to membrane proteins. Receptor-ligand complexes were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and visualized by autoradiography as described in 'Methods.' A representative autoradiograph is shown, indicating the presence of type I, type II, and type III TGF-β receptors. SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.](http://hyper.ahajournals.org/)
when VSMC from SHR and WKY rats are stimulated by growth factors raises the possibility that alterations in TGF-β1 signaling pathways account for the differences in growth properties of VSMC from the two rat strains.

Acknowledgments

This study was supported by a grant-in-aid from the National Heart Foundation of Australia. We thank Zymogenetics for their generous gift of PDGF-BB. We also thank Julie Simpson for assisting with the preparation of this manuscript.

References

Transforming growth factor-beta 1 gene activation and growth of smooth muscle from hypertensive rats.
A Agrotis, J Saltis and A Bobik

Hypertension. 1994;23:593-599
doi: 10.1161/01.HYP.23.5.593

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/23/5/593