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# Manidipine regulates the transcription of cytokine genes

**(platelet-derived growth factor/protein kinase C/calcium)**

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**ABSTRACT** **Manidipine, a $Ca^{2+}$-channel blocker, at concentrations that lower elevated blood pressure, modulates the transcription rates of cytokine genes in the mesangial cells of humans that had been stimulated with platelet-derived growth factor BB isomer; although the transcription for mRNA of interleukin 1β and granulocyte/monocyte colony-stimulating factor was inhibited, the transcription of mRNA for interleukin 6 was enhanced. Additionally, the induction of c-*fos*, c-*jun*, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase transcription was inhibited by manidipine. We conclude that manidipine, at nanomolar concentrations, is efficacious in modulating gene transcriptions that are involved in proinflammatory changes of mesangial cells. Thus, manidipine, at pharmacological concentrations that are one to two orders of magnitude lower than those required for inhibition of agonist- or depolarization ($K^+$)-induced vasoconstriction, causes changes in the activity of the genes that code for inflammatory mediators.**

Mesangial cells (MCs) proliferate during the course of immune- and nonimmune-mediated glomerular diseases of the kidney (1). There is evidence that platelet-derived growth factor (PDGF) is involved in inflammatory changes associated with renal tissue injury: receptors for PDGF have been identified in rat and human MCs (2); MCs express mRNAs for PDGF AA and BB isoforms (PDGF-AA and PDGF-BB) (2) and proliferate in response to PDGF (3); the expression of the receptors for PDGF and the mRNA of PDGF-BB in the kidney are increased during inflammation (4–6); and PDGF induces the transcription of various genes—e.g., low density lipoprotein receptor (LDL-R) and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase genes (7)—both of which are involved in the regulation of the biosynthesis of cellular cholesterol, which is impaired in glomerular diseases. In addition, it has been suggested that the proliferation of vascular smooth muscle cells (VSMCs) in the areas of plaque formation in atherosclerosis is due to PDGF being released at the sites of endothelial injury (8). Because PDGF has a similar role in atherosclerosis and glomerulosclerosis, the two diseases, consequently, are strikingly analogous (9).

$Ca^{2+}$-channel blockers lower elevated blood pressure (10), inhibit the synthesis of DNA in VSMCs (11) and MCs (12), and reduce the development of atherosclerotic lesions in experimentally induced hypercholesterolemia (13). They may increase the glomerular ultrafiltration coefficient, which is limited, in part, by MC function, and stimulate the expression of the interleukin 6 (IL-6) gene (14).

$Ca^{2+}$-channel blockers have protective effects on renal injury, as histological evidence has indicated (15). Furthermore, we have reported that PDGF-BB-dependent transcription of the genes for LDL-R and HMG-CoA reductase was modulated by the drugs: whereas PDGF enhanced the expression of the mRNA for LDL-R, the transcription of the mRNA of HMG-CoA reductase was inhibited (16). Moreover, the transcription of the latter gene was shown to involve the action of protein kinase C (PKC) (16).

To delineate the potential protective effect of $Ca^{2+}$-channel blockers on PDGF-mediated tissue injury, we have examined the potency of the $Ca^{2+}$-channel blocker manidipine for its ability to affect PDGF-BB-dependent transcription of mRNA for interleukin 1β (IL-1β), IL-6, and granulocyte/monocyte colony-stimulating factor (GM-CSF) in human MCs. We report here that (*i*) transcriptional rates of the mRNAs for IL-1β, c-*fos*, c-*jun*, HMG-CoA reductase, and GM-CSF are inhibited by the drug; (*ii*) this is paralleled by an inhibitory effect of manidipine on the synthesis of DNA and cell growth; (*iii*) in contrast, the transcriptional rate of the IL-6 gene is amplified; (*iv*) whereas the effects of the drug on gene transcription occur in the nanomolar range, the inhibitory effect on the synthesis of DNA and cell growth occurs at a 1- to 2-fold higher order of magnitude; and (*v*) the effect of manidipine on gene activation is apparently independent of the presence of $Ca^{2+}$ but involves the action of PKC.

## METHODS

**PDGF-BB.** PDGF-BB was obtained from Boehringer Mannheim. Its activity with respect to the proliferation of human MCs was dose dependent between 0.5 and 20 ng/ml.

**Human MCs.** Two primary human MC lines (H43, H44) were provided generously by Keith Stewart (Department of Internal Medicine, University of Aberdeen, U.K.). Cells were cultivated in RPMI 1640 medium (Fakola) supplemented with 10% fetal calf serum (FCS, GIBCO); 1% vitamin mix (GIBCO); 8 mM L-glutamine (GIBCO); and 10 ml of insulin/transferrin/selenite additive per liter (Sigma). Two days prior to experiments, $1 \times 10^6$ MCs were seeded into a 750-ml cell culture flask (Falcon); the culture medium was exchanged 4 hr later with RPMI 1640 medium supplemented with 0.1% FCS (low serum condition) to bring the cells to a quiescent state. The low serum medium was exchanged each day until the cells were stimulated by human recombinant PDGF-BB (rPDGF-BB; 10 ng/ml).

**Measurement of Mitogenicity.** The mitogenic effect was measured by the amount of [$^3$H]thymidine incorporated into DNA of human MCs according to the procedure of Chesterman *et al.* (17) and by assessment of cell proliferation as described by Lindl and Bauer (18). In brief, $1 \times 10^5$ quiescent cells were seeded into a 25-ml cell culture bottle (Falcon) and kept in low serum medium (0.1% FCS). On the following day, the cells were preincubated for 3 hr with manidipine ($1 \times 10^{-8}$

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Abbreviations: LDL-R, low density lipoprotein receptor; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-coenzyme A; PDGF, platelet-derived growth factor; rPDGF-BB, recombinant PDGF BB isomer; PKC, protein kinase C; VSMC, vascular smooth muscle cell; MC, mesangial cell; GM-CSF, granulocyte/monocyte colony-stimulating factor; IL-1β, interleukin 1β; IL-6, interleukin 6; PMA, phorbol 12-myristate 13-acetate; FCS, fetal calf serum.

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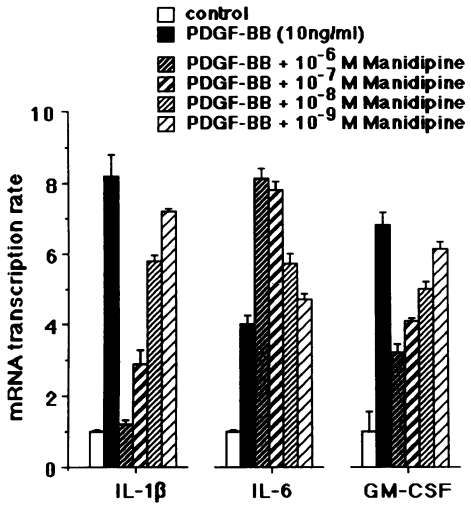


FIG. 1. Effects of various concentrations of manidipine on PDGF-BB-induced maximal transcriptional rates of IL-1$\beta$ (at 1 hr), IL-6 (at 4 hr), and GM-CSF (at 4 hr) genes in human MCs. Values are expressed as mean ± SD.

M) followed by stimulation with PDGF-BB (10 ng/ml) or incubated with low serum medium alone. The medium was replaced each day, and the cells were counted at days 1, 3, and 5. The experiments were performed in triplicate using both cell lines.

**Extraction of Total RNA.** Total RNA was extracted according to a modified protocol of Chomczynski and Sacchi (19), as described in detail (7). The RNA was quantitated by spectrophotometry (260 nm/280 nm).

**Northern Blot Analyses.** For Northern blots, equal amounts of RNA were denatured at 95°C for 5 min in sample buffer (50% formamide/10% glycine/1% bromophenol blue) and then size-fractionated by electrophoresis through a 1% agarose gel containing 0.02 M morpholinosulfonate, 2% formaldehyde, and 0.2 $\mu$g of ethidium bromide per ml (20) and performed as described (7).

**Nuclear Runoff Assay.** Nuclear runoff assays were performed as described by Nevins (21). Nuclei from $1 \times 10^8$ MCs were isolated, and RNA chains were elongated with creatinine kinase (5 mg/ml) in the presence of 500 mCi of [$^{32}$P]UTP (3000 mCi/mmol; 1 Ci = 37 GBq) and 10 mM ATP, CTP, and GTP. Labeled RNA was extracted according to the guanidinium isothiocyanate method and analyzed by dot blot (21). Quantitation of the synthesis *de novo* of mRNAs was assessed using autoradiography.

**Chemical Agents.** Manidipine was from Takeda (Kyoto), verapamil was from Knoll (Liestal, Switzerland), amlodipine was from Pfizer (Sandwich, U.K.), and diltiazem was from Gödecke (Freiburg, F.R.G.). The oligonucleotides for c-*fos*, c-*jun*, IL-1$\beta$, IL-6, and GM-CSF were purchased from British Biotechnology Laboratory (Abingdon, U.K.). The HLA-$\beta$ and the HMG-CoA reductase cDNA probes were obtained from the American Type Culture Collection. A23187 and TMB-8 were obtained from Aldrich (Steinheim, F.R.G.), and BayK 8644 was from Bayer (Leverkusen, F.R.G.).

## RESULTS

**Effects of Manidipine on rPDGF-BB-Induced Mitogenicity of MCs.** rPDGF-BB (10 ng/ml) stimulated the incorporation of [$^3$H]thymidine into DNA and the proliferation of MCs. The effect was dose dependent but was abrogated in the presence of manidipine; the $IC_{50}$ for DNA synthesis after 24 hr of incubation was $4 \times 10^{-8}$ M, and that for cell proliferation, determined after 3 days, was $6 \times 10^{-8}$ M.

**Effects of Manidipine on rPDGF-BB-Dependent Induction of IL-1$\beta$, IL-6, and GM-CSF mRNAs.** rPDGF-BB, when added to cultured MCs, induced the cytoplasmic accumulation of the mRNA transcripts of the three cytokines; however, the kinetics of the transcriptions were unique. Whereas transcription of IL-1$\beta$ mRNA began to increase at 30 min, peaking at 1 hr, and declining thereafter, the transcription of the mRNA for IL-6 increased at 2 hr and ceased at 4 hr; the transcriptional rate of the mRNA for GM-CSF started to increase at 1 hr and reached a maximum at 4 hr, after which it returned to its basal level. However, manidipine (at $10^{-9}$–$10^{-6}$ M) modified the rPDGF-dependent transcription of the genes in the following way: whereas transcription for IL-1$\beta$ was inhibited to the basal level, transcription of the mRNA for GM-CSF was inhibited by ≈50%. In contrast, the tran-

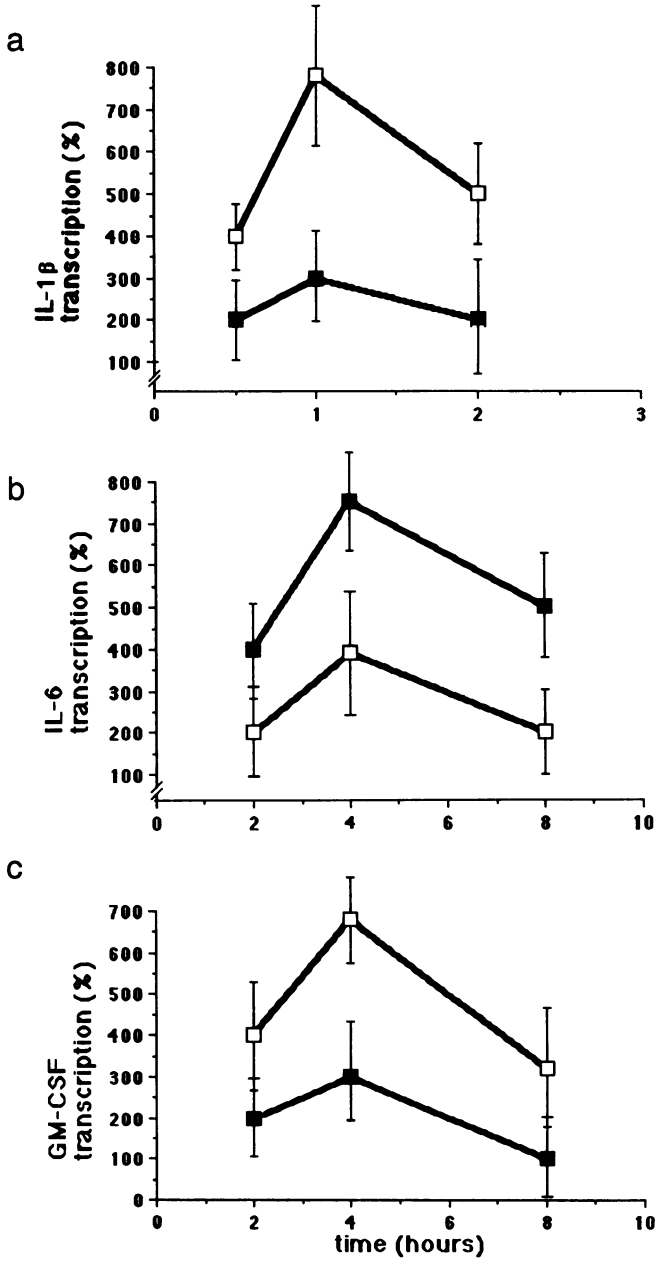


FIG. 2. Nuclear runoff analysis of MCs treated with 10 ng of PDGF-BB per ml (□) in the presence and absence of manidipine ($1 \times 10^{-8}$ M; ■). Transcription for IL-1$\beta$, IL-6, and GM-CSF RNAs is expressed as percent of the control gene (HLA-$\beta$). Data were from two MC cell lines, representing the mean ± SD of four independent measurements.

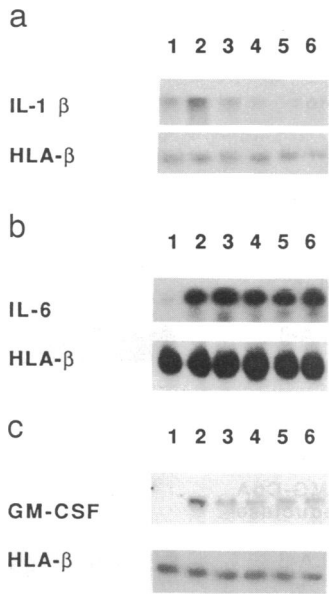


FIG. 3. Electrophoretic analysis of the effects of four $Ca^{2+}$-channel blockers on PDGF-BB-induced transcription; data were obtained at the times of maximal transcription of IL-1$\beta$ (*a*), IL-6 (*b*), and GM-CSF (*c*) in human MCs. Lanes 1, unstimulated control cells; lanes 2, effect of 10 ng of human rPDGF-BB per ml on gene transcriptional rates. To determine the effect of $Ca^{2+}$-channel blockers, MCs ($1 \times 10^6$), in a quiescent state, were preincubated with manidipine ($10^{-8}$ M) (lanes 3), verapamil ($10^{-8}$ M) (lanes 4), diltiazem ($10^{-8}$ M) (lanes 5), and amlodipine ($10^{-8}$ M) (lanes 6) for 3 hr prior to stimulation with PDGF-BB (10 ng/ml). Cells were incubated with $Ca^{2+}$-channel blockers for 3 hr after which PDGF-BB was added to the cells. HLA-$\beta$ gene transcription was expressed constitutively in human MCs.

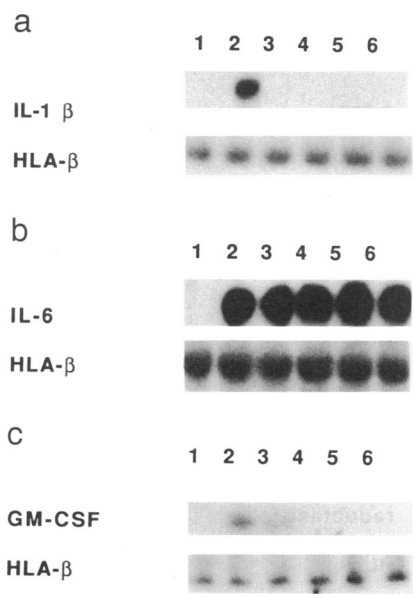


FIG. 4. Role of $Ca^{2+}$ on the effect of manidipine on PDGF-BB-induced gene transcription: electrophoretic analysis of IL-1$\beta$ (*a*), IL-6 (*b*), and GM-CSF (*c*) mRNAs in human MCs, determined at the times of maximal transcription. Lanes 1, unstimulated cells; lanes 2, stimulation with PDGF-BB (10 ng/ml). The concentration of intracellular calcium was changed by pretreatment of the cells prior to exposure to manidipine ($10^{-8}$ M) and PDGF-BB with 10 $\mu$M TMB-8 (lanes 3), 1 $\mu$M BayK 8644 (lanes 4), 10 $\mu$M $Ca^{2+}$ ionophore (lanes 5), and $Ca^{2+}$-free medium (lanes 6). HLA-$\beta$ gene transcription was expressed constitutively in human MCs.

scription of the IL-6 gene was augmented. Thus, manidipine ($10^{-8}$ M) increased the PDGF-dependent transcription of the mRNA by 1.5-fold (Fig. 1).

Transcripts formed from activation of several cytokines are subject to rapid degradation. To determine whether manidipine affects mRNA levels by modulating degradation of the transcripts, nuclear runoff transcription assays were performed. As shown in Fig. 2, manidipine affected the mRNAs at the level of transcription and not by changing their degradation; the increase of steady-state level of transcribed mRNA for IL-6 was abolished by treating the cells with actinomycin D (5 $\mu$g/ml). This confirms that the effect of manidipine on PDGF-BB-induced gene transcriptions occurred at the level of mRNA synthesis.

The viability of MCs treated with manidipine was ≈95% at 24 hr of culture; cells, following a cycle of washing and reexposure to rPDGF-BB, responded normally in terms of the synthesis of DNA and cell growth. In addition, the morphology of the cells was not altered by treating them with manidipine, as attested by phase-contrast microscopy.

**Effects of Various $Ca^{2+}$-Channel Blockers on rPDGF-BB-Induced Transcription of IL-1$\beta$, IL-6, and GM-CSF mRNAs.** Various $Ca^{2+}$-channel blockers affected PDGF-induced changes in the transcription of mRNA of these cytokines (Fig. 3). Manidipine, verapamil, diltiazem, and amlodipine, at $10^{-8}$ M, blocked the transcription of the IL-1$\beta$ gene to its basal level. Manidipine and verapamil, however, enhanced the transcription of mRNA for IL-6; diltiazem and amlodipine were ineffective. All four $Ca^{2+}$-channel blockers inhibited the transcription of the GM-CSF gene by 50%.

**Role of $Ca^{2+}$ Influx on the Effect of Manidipine on PDGF-Dependent Gene Activation.** The effect of manidipine on rPDGF-BB-induced gene activation is apparently not influenced by the presence or absence of $Ca^{2+}$. Thus, neither the addition of BayK 8644 (known to open $Ca^{2+}$ channels), the addition of TMB-8 (capable of blocking the release of $Ca^{2+}$ from internal depots), the omission of $Ca^{2+}$ from the incubation medium, nor the addition of the $Ca^{2+}$ ionophore A23187 led to a change in manidipine-induced modulation of PDGF-dependent gene transcription (Fig. 4). In the absence of PDGF, none of the three $Ca^{2+}$ modulators affected transcription of the genes studied (data not shown).

**Effect of Manidipine on rPDGF-BB-Induced Activation of c-*fos*, c-*jun*, and HMG-CoA Reductase mRNAs.** It was reported previously that the PDGF-dependent induction of c-*fos* and of HMG-CoA reductase genes is regulated by PKC (7, 22). Additionally, the PKC agonist phorbol 12-myristate 13-acetate (PMA) induces the transcription of the c-*jun* gene (23). A PDGF-dependent activation of PKC can be inhibited by various $Ca^{2+}$-channel blockers (11).

Similar to polymyxin B, an inhibitor of PKC, manidipine abolished PDGF-induced transcription of c-*fos* and HMG-CoA reductase mRNAs; transcription of c-*jun* mRNA, however, was reduced only partially (≈50%) (Fig. 5). This implies that manidipine affects gene-activating mechanisms by influencing the activity of PKC. Furthermore, the potency of $Ca^{2+}$-channel blockers to modulate gene transcription is distinct. Whereas all four drugs inhibited the transcription of c-*fos* and HMG-CoA reductase, the dihydropyridines, amlodipine and manidipine, reduced the induction of c-*jun* only partially (≈60%); conversely, the phenylalkylamine, verapamil, and the benzothiazepine, diltiazem, completely inhibited transcription of c-*jun* (Fig. 6).

## DISCUSSION

High concentrations of manidipine, at the micromolar range, inhibit growth and the synthesis of DNA in MCs; however, at much lower concentrations of the drug—i.e., in the nanomolar range—it lowers elevated blood pressure and blocks

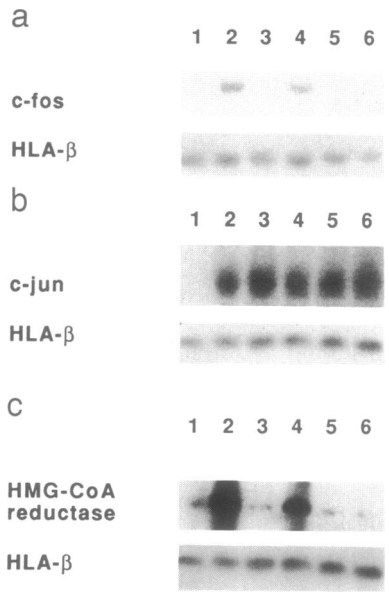


FIG. 5. Effect of manidipine on the PDGF-BB-induced gene transcription; Northern blot analysis of the c-*fos* (*a*), c-*jun* (*b*), and HMG-CoA reductase (*c*) mRNAs and the possible involvement of PKC. Data were obtained at the maximum level of gene transcriptions (c-*fos*, c-*jun* at 30 min; HMG-CoA reductase at 4 hr). Lanes 1, unstimulated control; lanes 2, MCs stimulated with 10 $\mu$M PMA; lanes 3, MCs preincubated with 10 $\mu$M polymyxin B followed by stimulation with PMA; lanes 4, MCs stimulated with 10 ng of PDGF-BB per ml; lanes 5, MCs preincubated with 10 $\mu$M polymyxin B followed by stimulation with 10 ng of PDGF-BB per ml; lanes 6, MCs preincubated with $1 \times 10^{-8}$ M manidipine followed by stimulation with 10 ng of PDGF-BB per ml. HLA-$\beta$ gene transcription was expressed constitutively in human MCs.

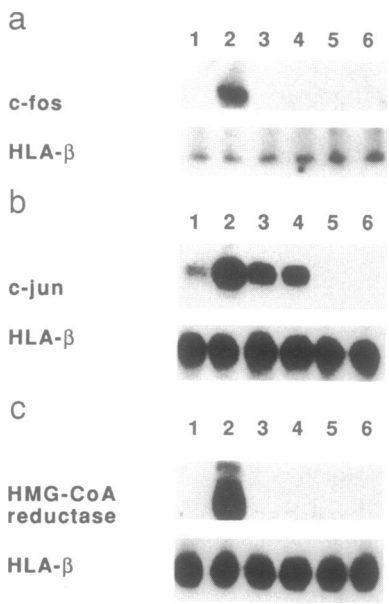


FIG. 6. Comparison of the inhibitory effect of four $Ca^{2+}$-channel blockers on PDGF-BB-induced transcription of c-*fos* (*a*), c-*jun* (*b*), and HMG-CoA reductase (*c*) in human MCs; Northern blot analysis determined at the times as indicated in legend to Fig. 5. Lanes 1, unstimulated control cells; lanes 2, effect of 10 ng of human PDGF-BB per ml on gene transcription rates. To determine the effect of $Ca^{2+}$-channel blockers, $1 \times 10^6$ MCs in a quiescent state were preincubated with $10^{-8}$ M manidipine (lanes 3), $10^{-8}$ M amlodipine (lanes 4), $10^{-8}$ M verapamil (lanes 5), and $10^{-8}$ M diltiazem (lanes 6) for 3 hr prior to stimulation with PDGF-BB (10 ng/ml). Cells were incubated with $Ca^{2+}$-channel blockers alone or PDGF-BB was added to the cells following a 3-hr preincubation. HLA-$\beta$ gene transcription was expressed constitutively in human MCs.

PDGF-BB-induced gene transcription of IL-1$\beta$ and GM-CSF while augmenting the induction of the transcription of the mRNA of IL-6. The effect of manidipine on gene activation of cytokines is apparently independent of $Ca^{2+}$ influx but coincides with inhibition of induction of c-*fos* and HMG-CoA reductase genes, all of which are regulated apparently by PKC. The inhibitory effect of manidipine at high concentrations on rPDGF-dependent growth and synthesis of DNA in MCs is similar to the antiproliferative potencies of diltiazem, nifedipine, and verapamil in MCs that were reported to be in the micromolar range (2, 11). However, those high concentrations of $Ca^{2+}$-channel blockers are not present usually under physiological conditions; they inhibit agonist- and depolarization ($K^+$)-induced contractions of VSMC preparations (24). However, our current data indicate that manidipine, at concentrations that are in the therapeutic range (nanomolar), is capable of lowering elevated blood pressure *in vivo* and modulates transcription of genes involved in the inflammatory response of MCs.

MCs are known to translate the mRNAs for IL-1$\beta$, IL-6, and GM-CSF, all of which are potentially relevant for inflammation and immunity at the glomerular site (25–27). The inhibition of transcription for IL-1$\beta$ and GM-CSF by manidipine indicates, therefore, its antiinflammatory potency by interfering with PDGF-dependent signals that otherwise induce autocrine and paracrine effects on MCs. Manidipine superinduced transcriptional amplification of the IL-6 gene that was comparable to the effect that verapamil had on human T cells (14). Considering that IL-6 possesses antiviral activity (28) and stimulates the expression of acute-phase proteins (28), these effects of manidipine on the genes involved in inflammation may be regarded as being protective. How can the dual effect of manidipine on gene expression be explained at a molecular level? The addition of neither BayK 8644, $Ca^{2+}$ ionophore A23187, $Ca^{2+}$-free medium, nor TMB-8 altered the effect of manidipine on PDGF-induced gene activation. This clearly argues against a $Ca^{2+}$-dependent mechanism relevant for the regulatory effect of manidipine on gene transcription. Although inhibition of ATP utilization and blockage of ($K^+$) channels cannot be excluded, we particularly favor the participation of PKC in gene regulation as relevant for the action of manidipine because (*i*) PDGF stimulates the activation of PKC (11); (*ii*) similarly, PDGF induces the expression of c-*fos* and HMG-CoA reductase, all of which are known to be stimulated by PKC; (*iii*) the induction of gene transcriptions is blocked by manidipine; and (*iv*) PDGF-dependent activation of PKC can be inhibited by $Ca^{2+}$-channel blockers (11).

Modulation of gene transcription is achieved by direct binding to specific DNA sequences or, indirectly, by altering the activity of transacting DNA-binding proteins. Assuming that PKC controls the interaction between repressor factors and its corresponding DNA sequences or DNA-binding proteins, respectively, a modulation of PKC activity would result in a change in the action of the repressor factors. In this context, it was reported that PMA, which stimulates the activity of PKC, causes the expression of c-*fos* and c-*jun* (29). Fos and Jun are able to form heterodimeric complexes (transcription factor AP-1) which bind to and activate genes via the PMA response element (for review, see ref. 30).

According to recent reports, the interaction of various growth factors and hormones affecting transcriptional events involves the action of AP-1 (31, 32). Moreover, c-*jun* encodes a protein similar to AP-1 (33). Thus, the PKC signal transduction pathway converges through the interaction between the cell membrane receptors and the Jun and Fos oncoproteins. Taking into account that $Ca^{2+}$-channel blockers intercalate into biomembranes, thereby changing hydrophobic/

hydrophilic interactions, manidipine, in addition to other $Ca^{2+}$-channel blockers, might affect the activity of PKC by influencing these signals with resultant changes at the gene level. The unique ability of $Ca^{2+}$-channel blockers to alter c-*jun*/AP-1 activation may provide a new way of defining its modulatory influence on gene activation. Alternatively, manidipine can bring about the stimulation of gene transcription—i.e., for IL-6—by inhibiting the synthesis of a negative regulatory protein that controls mRNA transcription (14, 16). Further studies are required to resolve the action of $Ca^{2+}$-channel blockers with regard to their organ-protective mechanisms at the gene level.

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1. Glassock, R. J., Cohen, A. H., Adler, S. & Ward, H. J. (1986) in *The Kidney*, eds. Brenner, B. & Rector, F. (Saunders, Philadelphia), pp. 929–1084.
2. Shultz, P. J., DiCorleto, P., Silver, B. J. & Abboud, H. E. (1988) *Am. J. Physiol.* **255,** F674–F684.
3. Abboud, H. E., Poptic, E. & DiCorleto, P. (1987) *J. Clin. Invest.* **80,** 675–683.
4. Yoshimura, A., Gordon, K., Alpers, Ch. E., Floege, J., Pritzl, P., Ross, R., Couser, W. G., Bowen-Pope, D. F. & Johnson, R. J. (1991) *Kidney Int.* **40,** 470–476.
5. Gesulado, L., Abboud, H. E., Pinzani, M., Floriano, J. & Lamm, M. E. (1990) *Kidney Int.* **37,** 414 (abstr.).
6. Fellström, B., Klareskog, L., Heldin, C. H., Larsson, E., Rönnstrand, L., Terracio, L., Tufveson, G., Wahlberg, J. & Rubin, K. (1989) *Kidney Int.* **36,** 1099–1102.
7. Roth, M., Emmons, L. R., Perruchoud, A. & Block, L. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1888–1892.
8. Gordon, A. A., Ferns, A., Raines, E. W., Sprugel, K. H., Motani, A. S., Reidy, M. A. & Ross, R. (1991) *Science* **253,** 1129–1132.
9. Diamond, J. R. & Karnovsky, M. J. (1988) *Kidney Int.* **33,** 917–924.
10. Sunderrajan, S., Reams, G. & Bauer, J. H. (1986) *Hypertension* **8,** 238–242.
11. Block, L. H., Emmons, L. R., Vogt, E., Sachinidis, A., Vetter, W. & Hoppe, J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2388–2392.
12. Shultz, P. J. & Raij, L. (1990) *Hypertension*, Suppl. I, **15,** I-76–I-80.
13. Godfraind, T., Albertini, A. & Paoletti, R. (1982) *Calcium Modulators* (Elsevier, Amsterdam).
14. Walz, G., Zanker, B., Barth, C., Wieder, K. J., Clark, S. C. & Strom, T. B. (1990) *J. Immunol.* **144,** 4242–4248.
15. Tolins, J. B., Hartich, H. B. & Raij, L. (1989) *Clin. Res.* **37,** 503A (abstr.).
16. Block, L. H., Matthys, H., Emmons, L. R., Perruchoud, A., Erne, P. & Roth, M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9041–9045.
17. Chesterman, C. N., Walker, T., Grego, B., Chamberlain, K., Hearn, M. B. & Morgan, F. J. (1983) *Biochem. Biophys. Res. Commun.* **116,** 908–916.
18. Lindl, T. J. & Bauer, T. (1989) *Zell- und Gewebekultur* (Fischer, Stuttgart, F.R.G.), 2nd Ed.
19. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Nevins, J. R. (1987) *Methods Enzymol.* **152,** 234–241.
22. Mazzone, T., Basheeruddin, K., Ping, L., Frazer, S. & Getz, G. S. (1989) *J. Biol. Chem.* **264,** 1787–1792.
23. Sherman, M. L., Stone, R. M., Dattar, R., Bernstein, S. H. & Kufe, D. W. (1990) *J. Biol. Chem.* **265,** 3320–3323.
24. Godfraind, T., Miller, R. & Wibo, M. (1986) *Pharmacol. Rev.* **38,** 321–416.
25. Lovett, D. H. & Larsen, A. (1988) *J. Clin. Invest.* **82,** 115–122.
26. Ruef, C., Budde, K., Lacy, J., Northemann, W., Baumann, M., Sterzel, W. & Coleman, D. L. (1990) *Kidney Int.* **38,** 249–257.
27. Mori, T., Bartocci, A., Satriano, J., Santiago, A. & Schlöndorff, D. (1990) *Kidney Int.* **37,** 424 (abstr.).
28. Gauldi, J., Richards, C., Harnish, D., Landsorp, P. & Baumann, H. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7251–7255.
29. Haas, R., Brach, M., Kharbanda, S., Giese, G., Traub, P. & Kufe, D. (1991) *J. Cell Physiol.* **149,** 125–131.
30. Schüle, R. & Evans, R. M. (1991) *Trends Genet.* **7,** 377–381.
31. Chedid, M., Yoza, B. K., Brooks, J. W. & Mizel, S. E. (1991) *J. Immunol.* **147,** 867–873.
32. Ryder, K. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8464–8467.
33. Angel, P., Allegretto, E. A., Okino, S. T., Hattori, K., Boyle, W. J., Hunter, T. & Karin, M. (1988) *Nature (London)* **332,** 166–171.