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ANGIOTENSIN II INDUCES EXPRESSION OF THE C-FOS GENE THROUGH PROTEIN KINASE C ACTIVATION AND CALCIUM ION MOBILIZATION IN CULTURED VASCULAR SMOOTH MUSCLE CELLS[1]

Yasuhiro Kawahara[2], Michitoshi Sunako, Terutaka Tsuda, Hisashi Fukuzaki, Yasuo Fukumoto* and Yoshimi Takai*

Departments of Internal Medicine (1st Division) and Biochemistry*, Kobe University School of Medicine, Kobe 650, Japan

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SUMMARY: Incubation of the serum-deprived cultures of rat vascular smooth muscle cells with angiotensin II, a potent vasoconstrictor, caused a rapid and transient increase in the c-fos mRNA level. The doses of this agonist necessary for the increase in the c-fos mRNA level coincided with those for the phospholipase C-mediated hydrolysis of phosphoinositides. Moreover, protein kinase C-activating 12-O-tetradecanoylphorbol-13-acetate and Ca^{2+}-ionophore A23187 increased the c-fos mRNA level in an additive manner. These results suggest that angiotensin II induces expression of the c-fos gene through the activation of protein kinase C and Ca^{2+} mobilization in cultured vascular smooth muscle cells.

It is important to clarify the intracellular mechanisms of proliferation of vascular SMC to understand the processes of development of atherosclerosis (1). Recently, Kindy and

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[2]To whom all correspondence should be addressed.

Abbreviations used are: SMC, smooth muscle cells; FCS, fetal calf serum; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; TPA, 12-O-tetradecanoylphorbol-13-acetate; DMEM, Dulbecco's modified Eagle's medium; IP_1, inositol-1-monophosphate; IP_2, inositol-1,4-bisphosphate; IP_3, inositol-1,4,5-trisphosphate; EGF, epidermal growth factor.

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Sonenshein (2) have described that expression of two proto-oncogenes, the c-fos and c-myc genes, may be involved in the FCS-induced proliferation of calf vascular SMC. The expression of these two genes has been implicated in the PDGF-induced transition from the G_0 to G_1 phase of the cell cycle in BALB/c 3T3 cells (for a review, see Ref. 3). We have previously shown that protein kinase C and Ca^{2+} are independently involved in the PDGF- and FGF-induced expression of these genes in Swiss 3T3 cells (4-6). These two intracellular messenger systems are regulated by the phospholipase C-mediated hydrolysis of phosphoinositides which is linked to the receptors for PDGF and FGF in this cell type (7-9).

Angiotensin II is a potent vasoconstrictor which plays a crucial role in the pathogenesis of some forms of hypertension (for a review, see Ref. 10). This vasoconstrictor induces the phospholipase C-mediated hydrolysis of phosphoinositides in vascular SMC (11-13). It is well established that Ca^{2+} is essential for vasoconstriction, although the definitive role of protein kinase C in this function is not clear at present. Interesting evidence has recently been presented that PDGF induces vasoconstriction in addition to cell proliferation in vascular SMC (14). This observation has promptly raised the question as to whether vasoconstricting angiotension II can inversely induce the expression of the c-fos or c-myc gene which is implicated in the regulation of cell proliferation.

This communication reports for the first time that angiotensin II can induce expression of the c-fos gene through the activation of protein kinase C and Ca^{2+} mobilization in cultured rat aortic SMC.

EXPERIMENTAL PROCEDURES

A pBR322 plasmid containing the v-fos gene (pfos-1) was a generous gift from Drs. T. Sugiyama and R. Takahashi (Kobe

University School of Medicine, Kobe, Japan) who originally obtained it from Dr. I.M. Verma (Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, U.S.A.). Angiotensin II was a generous gift from Ciba-Geigy Ltd., Basle, Switzerland. TPA and Ca^{2+} ionophore A23187 were purchased from CCR Inc. and Calbiochem, respectively. Myo-[2-^{3}H]inositol (16.3 Ci/mmol) and [α-^{32}P]dCTP (6,000 Ci/mmol) were obtained from Amersham Japan, Tokyo, Japan. Other materials and chemicals were obtained from commercial sources. Rat aortic SMC were kindly supplied by Dr. Y. Hirata (National Cardiovascular Center Research Institute, Suita, Japan) who prepared them from the explants of the thoracic aortae of 9-week old male Wistar rats as described by Ross (15). The cells were cultured in DMEM supplemented with 10% FCS, 100 µg/ml of streptomycin and 100 units/ml of penicillin at 37°C in a humidified atomosphere of 95% air: 5% CO_2. For experiments, the cells between passage levels 8 and 15 were seeded into 35-mm dishes for the measurement of the production of inositol phosphates and into 60-mm dishes for the measurement of c-fos mRNA at a density of 2 x 10^4 cells/cm^2, refed after 2 days, and used 5 days after the change of medium. The production of inositol phosphates was measured as described by Alexander et al. (16) except that inositol-free DMEM was used during the labeling of the cells with myo-[2-^{3}H]inositol. The water-soluble inositol phosphates were separated by Dowex AG1-X8 column chromatography as described by Berridge et al. (17). Total RNA was extracted from the cells as described by Chirgwin et al. (18) using CsCl gradient centrifugation. c-fos mRNA was analyzed by Northern blotting or dot blotting as described previously (19). The levels of c-fos mRNA were expressed as cpm/4 µg of total RNA.

RESULTS

Incubation of quiescent cultures of rat vascular SMC with FCS caused a rapid and transient increase in the c-fos mRNA level as estimated by Northern blot analysis (Fig. 1). c-fos mRNA reached a maximal level at 30 min after the addition of FCS, followed by a decline to the basal level. This result agrees with that described previously (2). Under the same conditions, angiotensin II also increased the c-fos mRNA level and the maximal level obtained by angiotensin II was comparable to that obtained by FCS (Fig. 1).

Fig. 2 shows that angiotensin II induces the accumulation of IP_1, IP_2 and IP_3 in a time-dependent manner. The increases in these inositol phosphates were detectable at 5 sec after the addition of angiotensin II. IP_1 progressively increased during a 5-min incubation with this agonist. IP_2 reached a maximal level between 30 sec and 1 min. The angiotensin II-induced increase in IP_3 was biphasic. The physiological significance of the biphasic

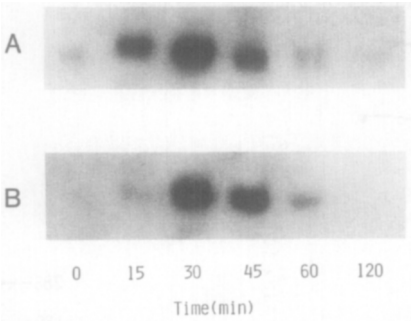


Fig. 1. FCS- or angiotensin II-induced increase in c-fos mRNA. The cultures were incubated with FCS (10%) or angiotensin II (1 μM) for various periods of time at 37°C. The c-fos mRNA level was analyzed by Northern blotting. A, with FCS; B, with angiotensin II.

increase in IP_3 is not known at present. It is likely that IP_1, IP_2 and IP_3 are derived from the phospholipase C-mediated hydrolysis of the respective phosphoinositides as described in many other cell types (for a review, see Ref. 20).

The dose-response curves of angiotensin II for the increase in the c-fos mRNA level and the phospholipase C reactions are compared in Fig. 3. In this figure, the extent of the phospholipase C reactions is represented by the production of IP_3, since the doses of angiotensin II necessary for the production of IP_1,

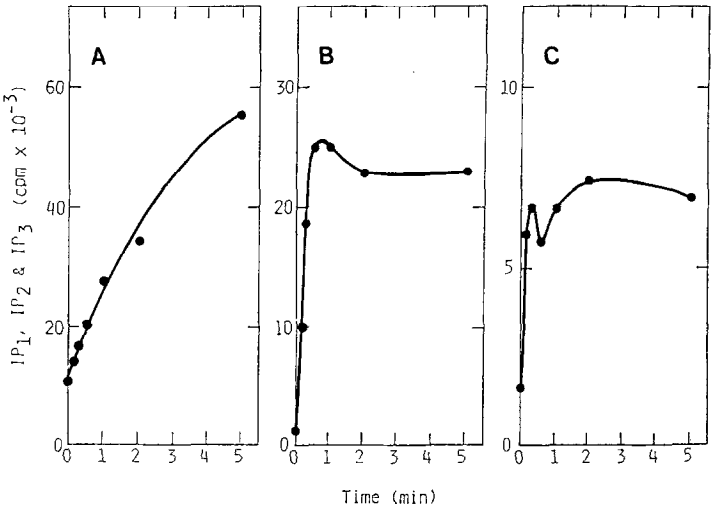
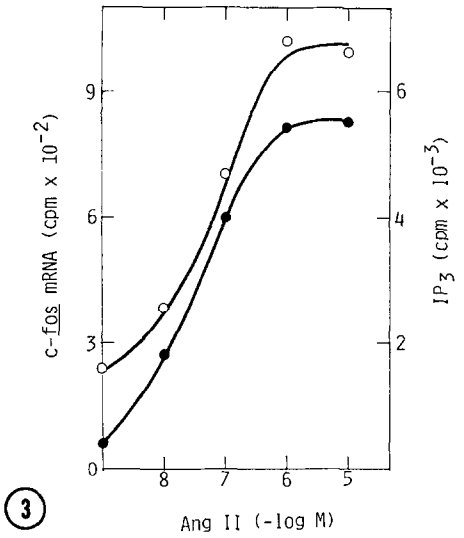


Fig. 2. Time courses of the angiotensin II-induced increase in inositol phosphates. The cultures, prelabeled with myo-[2-^{3}H]inositol, were incubated with angiotensin II (1 μM) for various periods of time at 37°C. A, IP_1; B, IP_2; C, IP_3. Each value is the mean of triplicate determinations.



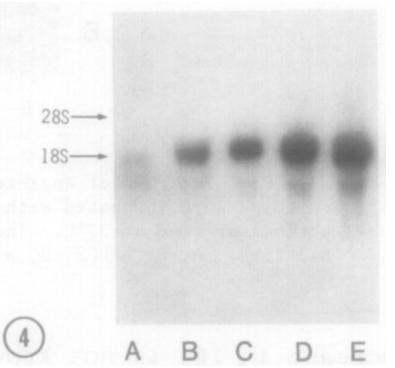


Fig. 3. Dose-response curves of angiotensin II for the increase in c-fos mRNA and the production of IP_3. The cultures were incubated with various doses of angiotensin II for 30 min for the measurement of the c-fos mRNA level and for 15 sec for the measurement of IP_3. The c-fos mRNA level was analyzed by dot blotting. (●—●), c-fos mRNA; (○—○), IP_3. Each value is the mean of three different experiments. Ang II indicates angiotensin II.

Fig. 4. TPA- or Ca^{2+} ionophore A23187-induced increase in c-fos mRNA. The cultures were incubated for 30 min at 37°C. TPA (40 nM), A23187 (1 μM) and angiotensin II (1 μM) were added as indicated. The c-fos mRNA level was analyzed by Northern blotting. A, No addition; B, with TPA; C, with A23187, D, with TPA plus A23187; E, with angiotensin II.

IP_2 and IP_3 were essentially the same. The doses of this agonist necessary for the increase in the c-fos mRNA level were similar to those for the production of IP_3.

In the last set of experiments, the effect of protein kinase C-activating TPA and Ca^{2+} ionophore A23187 on the c-fos mRNA level was examined. Either TPA or Ca^{2+} ionophore A23187 alone raised the c-fos mRNA level (Fig. 4). The increase in c-fos mRNA induced by these agents was also transient and maximal levels were obtained at 30 min (data not shown). Quantitative results given in Fig. 5 show that both TPA and Ca^{2+} ionophore A23187 increased the c-fos mRNA level in a dose-dependent manner. Although the maximal level of the c-fos mRNA obtained by TPA or Ca^{2+} ionophore A23187 was about one third of that obtained by angiotensin II, TPA and Ca^{2+} ionophore A23187 increased the c-fos mRNA level in an additive manner (Figs. 4 and 5).

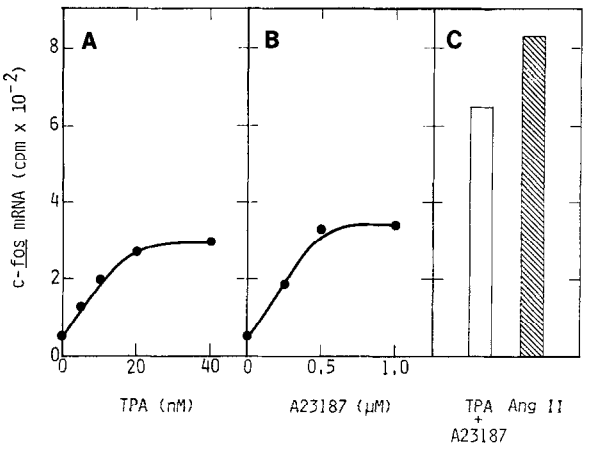


Fig. 5. Increase in c-fos mRNA by TPA and Ca^{2+} ionophore A23187. The cultures were incubated for 30 min at 37°C. TPA, A23187 and angiotensin II were added as indicated. The c-fos mRNA level was analyzed by dot blotting. A, with TPA; B, with A23187; C, with TPA (40 nM) plus A23187 (1 μM) or with angiotensin II (1 μM). Each value is the mean of three different experiments. Ang II indicates angiotensin II.

DISCUSSION

This paper has shown that angiotensin II, one of the potent vasoconstrictors, increases the c-fos mRNA level in cultured rat aortic SMC. It is likely that angiotensin II stimulates, at least partly, expression of this gene as described for FCS (2), although the possibility that the increase in c-fos mRNA is due to the stabilization of the mRNA can not be neglected. It has previously been shown that angiotensin II induces the phospholipase C-mediated hydrolysis of phosphoinositides which is coupled with the activation of protein kinase C and Ca^{2+} mobilization in rat vascular SMC (11-13). We have confirmed this observation and shown that the doses of angiotensin II necessary for the phospholipase C reactions and the increase in the c-fos mRNA level are similar. Moreover, protein kinase C-activating TPA and Ca^{2+} ionophore A23187 increase the c-fos mRNA level in an additive manner. These results strongly suggest that vasoconstricting angiotensin II induces expression of the c-fos gene through the two intracellular messenger systems, protein kinase C and Ca^{2+}, as

described for growth factors such as PDGF and FGF in Swiss 3T3 cells (6).

SMC are the major cellular constituent of the medial layer of the blood vessels and proliferation of these cells plays a key role in the progression of atherosclerosis. Some growth factors such as PDGF and EGF are thought to be responsible for the proliferation of vascular SMC in the lesion of atherosclerosis (1). Although the effect of angiotensisn II on the proliferation of vascular SMC has to be examined, the fact that the c-fos gene is expressed in rat aortic SMC in response to angiotensin II tempts us to speculate that angiotensin II may act as a growth factor for vascular SMC in addition to acting as a vasoconstrictor, since expression of this gene has been implicated in the G_0 to G_1 transition in BALB/c 3T3 cells (3). It is well known that hypertension is one of the major risk factors of atherosclerosis. Our present observation may explain the relationship between some forms of hypertension and accelerated atherosclerosis.

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