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Role of Tyrosine Kinases in Vascular Contraction in Deoxycorticosterone Acetate-Salt Hypertensive Rats

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It has been known that activation of tyrosine kinases is involved in signal transduction. Role of the tyrosine kinase in vascular smooth muscle contraction was examined in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Male Sprague-Dawley rats underwent uninephrectomy, one week after which they were subcutaneously implanted with DOCA (200 mg/kg) and supplied with 1% NaCl and 0.2% KCl drinking water for 4~6 weeks. Control rats were treated the same except for that no DOCA was implanted. Helical strips of carotid arteries were mounted in organ baths for measurement of isometric force development. Genistein was used as a tyrosine kinase inhibitor. Concentration-response curves to 5-hydroxytryptamine (5-HT) shifted to the right by genistein in both DOCA-salt hypertensive and control rats. Although the sensitivity to genistein was similar between the two groups, the maximum force generation by 5-HT was less inhibited by genistein in arteries from DOCA-salt hypertensive rats than in those from controls. Genistein-induced relaxations were attenuated in arteries from DOCA-salt rats. Genistein affected the contraction to phorbol 12, 13-dibutyrate (PDBu) neither in DOCA-salt nor in control arteries. These observations suggest that tyrosine kinase is involved in 5-HT-induced vascular contraction, of which role is reduced in DOCA-salt hypertension.

Key Words: Tyrosine kinases, Vascular smooth muscle contraction, Deoxycorticosterone acetate-salt hypertensive rats

INTRODUCTION

Signal transduction associated with contraction of vascular smooth muscle is a complex process involving multiple regulatory mechanisms (van Breeman & Saida, 1989; Somlyo & Somlyo, 1990). Tyrosine kinases, enzymes which phosphorylate proteins on tyrosine residues, have been known to participate in diverse signalling pathways involving various neurotransmitters (Huganir & Greengard, 1990; O'Dell et al, 1991) and initiation of mitogenic responses to certain growth factors (Ullrich & Schlessinger, 1990; Bilder et al, 1991).

Previous studies have shown that the vascular smooth muscle contains unusually high levels of tyrosine kinase activity (Di Salvo et al, 1988; Di Salvo et al, 1989). Although the specific role of protein tyrosine kinases in vascular smooth muscle has not been fully understood, the activation of tyrosine kinases is involved in signal transduction leading to growth and differentiation. It is also shown that proliferation and gene expression can be blocked in vascular smooth muscle via specific inhibition of protein tyrosine kinase activity (Levitzki & Gilon, 1991). Furthermore, vasoconstriction and vascular smooth muscle cell proliferation may share common biochemical signalling pathways (Sauro & Thomas, 1993). In fact, tyrosine kinase inhibitor has been shown to inhibit epidermal growth factor (EGF)-induced vasoconstriction in rabbit aortic rings (Merkel et al, 1993).

On the other hand, 5-hydroxytryptamine (5-HT) exerts its action in smooth muscle contraction via activation of phospholipase C (Chaffoy de Courcelles

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et al, 1985; Nakaki et al, 1985), which leads to the hydrolysis of membrane phophoinositides. 5-HT has also been found to be related to the mitogenic action of smooth muscle cells, of which proliferating activity is enhanced in spontaneously hypertensive rats (Paquet et al, 1989). Therefore, it is speculated that tyrosine phosphorylation other than phosphoinositide metabolism participates in 5-HT-induced vascular smooth muscle contraction and in the development of certain forms of hypertension.

This study was aimed to examine the role of tyrosine kinase in 5-HT-induced vasocontraction and to determine whether tyrosine phosphorylation is altered in a hypertensive state. Rats made deoxycorticosterone acetate (DOCA)-salt hypertensive were used.

METHODS

Animals

Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), male Sprague-Dawley rats (150~200 g) underwent left nephrectomy, they were given subcutaneous implantation of Silastic strips (silicone rubber) impregnated with DOCA (200 mg/kg) one week after the nephrectomy. Control rats received a sham treatment; a left nephrectomy was performed, and Silastic without DOCA was implanted. All animals received standard rat chow and 1% NaCl and 0.2% KCl in their drinking water for 4~6 weeks. Systolic blood pressure was recorded weekly by the tail-cuff method.

Tissue preparation

Carotid arteries were removed and placed in a dish containing cold physiological salt solution (PSS). They were cleaned of all connective tissues and cut into helical strips (0.8×10 mm) under a dissecting microscope. The endothelium was removed by a gentle rubbing with a cotton swab. The PSS used was of the following composition (in mmol/L): NaCl 130, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 14.9, Dextrose 5.5, $CaNa_2$ EDTA 0.026 and $CaCl_2$ 1.6. The strips were mounted vertically in a 10 mL organ bath containing PSS and connected to Grass FT03 transducers to measure their changes in isometric tension. The bath temperature was maintained at 37°C and the solution was aerated with a mixture of 95% O_2 and 5% CO_2 throughout the experiment. A passive tension (500 mg) was applied to the strips and 90 minutes were allowed to elapse for equilibration. During the equilibration period, the solution in the bath was changed several times, and the passive force on each arterial segment was readjusted to 500 mg.

Experimental protocol

Four arterial strips were placed in the muscle bath: two from DOCA-salt hypertensive rats and two from control rats. Cumulative concentration response curves to 5-HT (10^{-9} to 10^{-4} mol/L) were obtained. Contractile force was measured in milligrams in response to increasing concentrations of an agonist, and the result was expressed as a percentage of the maximal response.

To confirm the role of tyrosine kinase in vascular smooth muscle contractility, the strips were pretreated with genistein (5×10^{-6} mol/L), a tyrosine kinase inhibitor. Relaxations induced by genistein (10^{-8} to 3×10^{-5} mol/L) were calculated as percent reductions from the maximal tension obtained by ED_{50} of 5-HT. Spontaneous relaxations by time course were subtracted.

To determine whether genistein reacted specifically on tyrosine kinases, dose-response curves to phorbol 12, 13-dibutyrate (PDBu, 10^{-10} to 10^{-6} mol/L), protein kinase C activator, were obtained in arteries from DOCA-salt hypertensive and control rats in the absence and presence of genistein.

Drugs

5-HT was purchased from Sigma Chemical Company. PDBu was obtained from Biomolecular Research Laboratories and genistein was from LC Laboratories. All other chemicals were of reagent grade (Sigma).

Statistics

Values presented in the figures are expressed as the means and standard error of the means. ED_{50} was determined by a plot of the percentage of response against the log concentration of the agonist and expressed as a negative log molar (pD_2). Statistical comparisons between the groups were performed by Student's t test. A P value less than 0.05 was con-

sidered statistically significant.

RESULTS

The systolic blood pressure in DOCA-salt hypertensive rats at 4~6 weeks was significantly higher (177±3 mmHg, $P<0.01$) than that (119±2 mmHg) in control rats.

As shown in Fig. 1, 5-HT caused a dose-dependent contraction in isolated carotid strips. Carotid arteries from DOCA-salt hypertensive group were more sensitive to 5-HT than those from control group as evidenced by a leftward shift of the concentration-response curve (pD_2: 6.30±0.13 vs 5.74±0.11, $P<0.01$). The dose-response curve to 5-HT inhibited by genistein (5×10^{-6} mol/L) in both DOCA-salt hypertensive and control rats (pD_2: 5.88±0.12 vs 5.29±0.13, both $P<0.05$). The magnitude of the concentration shift was similar between the two groups (2.6 fold in DOCA vs 2.7 fold in control).

The effect of genistein on basal tension in arteries from control rats is shown in Fig. 2. Genistein caused an attenuation of isometric tension development in

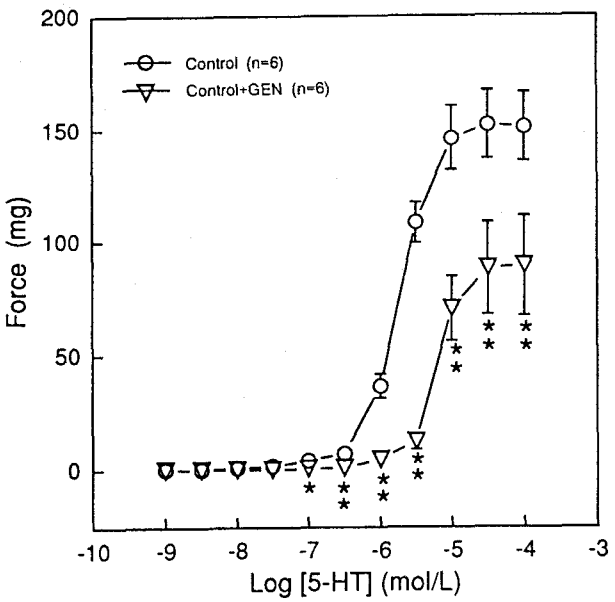

Fig. 2. Isometric forces developed in response to 5-hydroxytryptamine (5-HT) in the absence and presence of genistein (5×10^{-6} mol/L) for control carotid arteries. *$P<0.05$, ** $P<0.01$, significant difference from control without genistein.

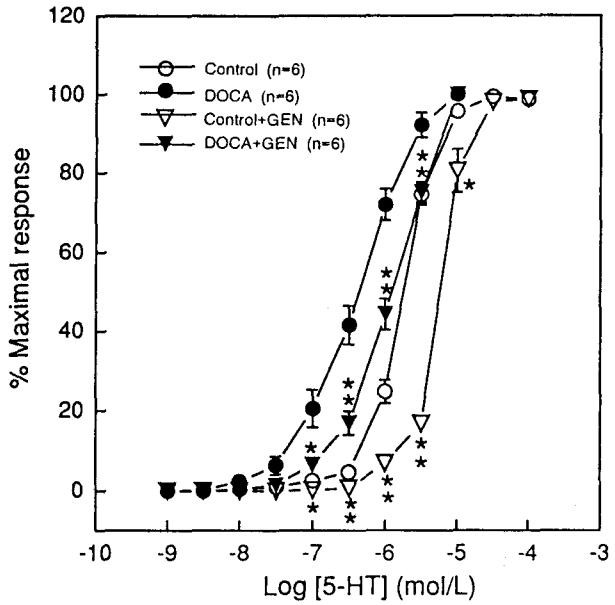

Fig. 1. Contractile responses to 5-hydroxytryptamine (5-HT) in the absence and presence of genistein (5×10^{-6} mol/L) for DOCA-salt hypertensive and control carotid arteries. *$P<0.05$, **$P<0.01$, significant difference from control without genistein.

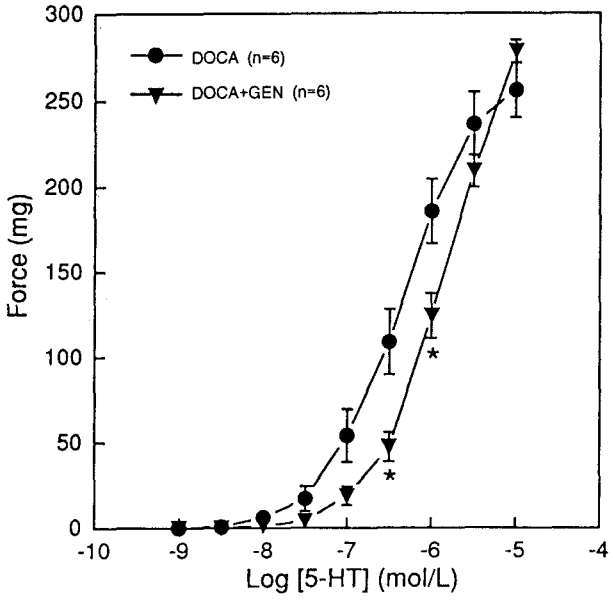

Fig. 3. Isometric forces developed in response to 5-hydroxytryptamine (5-HT) in the absence and presence of genistein (5×10^{-6} mol/L) for DOCA-salt hypertensive carotid arteries. *$P<0.05$, significant difference from control without genistein.

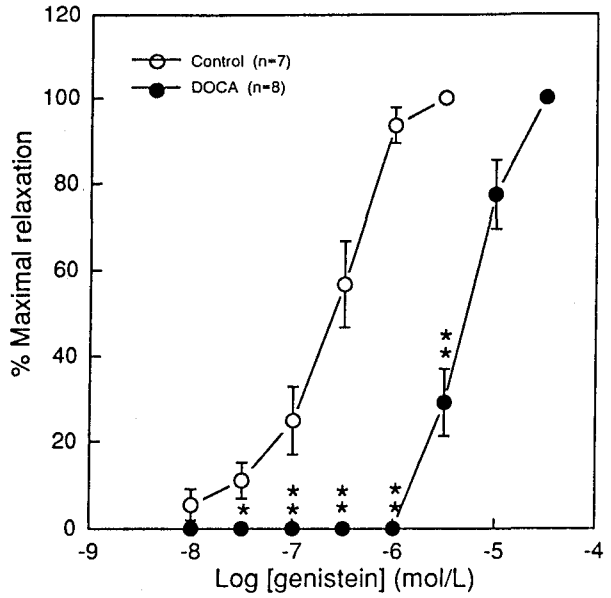

Fig. 4. Vasorelaxation responses to genistein. Contraction was maintained by 5-hydoxytryptamine (ED_{50}) for DOCA-salt hypertensive and control carotid arteries. *P <0.05, **P <0.01, significant difference from control.

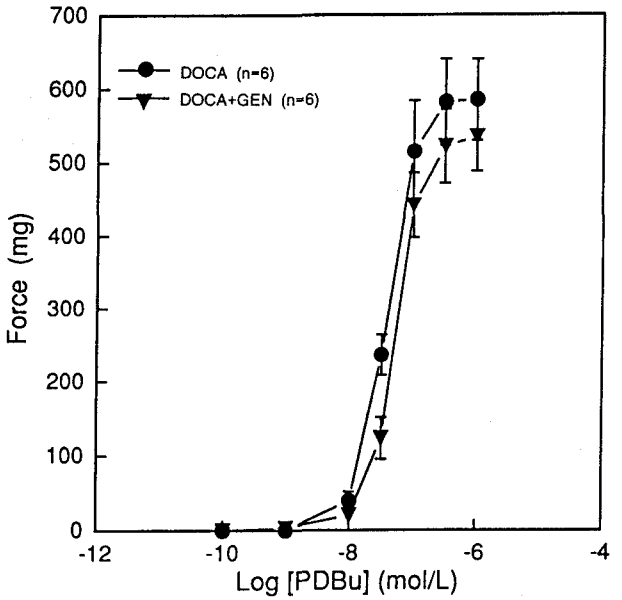

Fig. 6. Isometric forces developed in response to phorbol 12,13-dibutyrate (PDBu) in the absence and presence of genistein (5×10^{-6} mol/L) for DOCA-salt hypertensive carotid arteries.

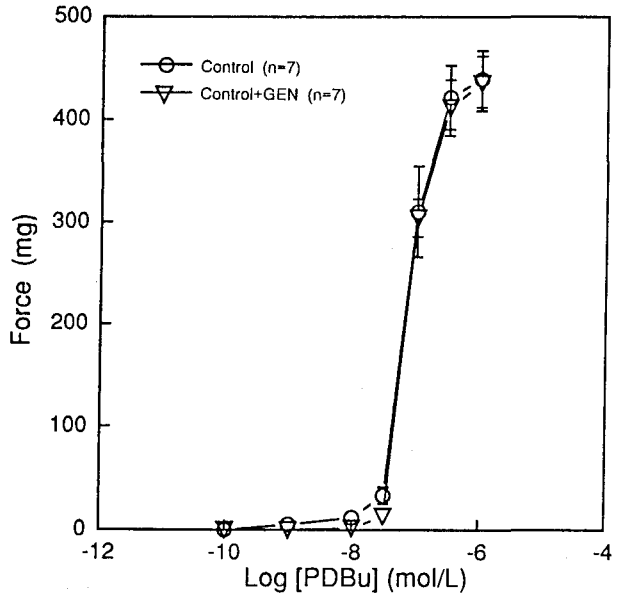

Fig. 5. Isometric forces developed in response to phorbol 12,13-dibutyrate (PDBu) in the absence and presence of genistein (5×10^{-6} mol/L) for control carotid arteries.

response to 5-HT. Maximal contraction to 5-HT was inhibited approximately 59%.

The force generated to 5-HT was inhibited less in arteries from DOCA-salt hypertensive rats as compared with that from control rats. Genistein did not affect the force development (109%) at the high concentration of 5-HT in DOCA-salt hypertensive rats (Fig. 3).

Fig. 4 depicts the relaxation response to genistein examined in carotid strips precontracted with an ED_{50} of 5-HT. The relaxation curve to genistein shifted to the right in arteries from DOCA-salt hypertensive rats. Genistein caused a relaxation in a dose-dependent fashion in control rats, while it was without effect at the lower concentrations in DOCA-salt hypertensive rats.

No significant changes in force generation to PDBu were obtained by genistein either in DOCA-salt hypertensive or in control rats (Fig. 5, 6).

DISCUSSION

Augmented vascular responsiveness to 5-HT is observed in various models of hypertension (Webb & Bohr, 1983). In the present study, the dose-contrac-

tion curves to 5-HT in carotid strips from DOCA-salt hypertensive rats shifted to the left as compared with control rats. Similar results have been obtained by Turla & Webb (1990) in genetically hypertensive rats. They speculated that the enhanced contractile responses to 5-HT are attributed to a greater release of intracellular calcium from a cellular pool, an augmented 5-HT-stimulated phosphoinositide hydrolysis and alterations in number or affinity of inositol trisphosphate receptors on the endoplasmic reticulum of vascular smooth muscle. In addition, it has been demonstrated that the contractile responses to 5-HT in calcium-free solution after loading of cellular stores of calcium are greater in DOCA-salt hypertensive rats than in normotensive rats (Mecca & Webb, 1984). Therefore, it is possible in DOCA-salt hypertension that an enhanced mobilization of intracellular calcium may be responsible for the increase in vascular reactivity to 5-HT.

It has been shown that vasoactive agents such as 5-HT, angiotensin-II and bradykinin enhance cell proliferation (Paquet et al, 1989). These findings suggest that 5-HT may act on receptors which contain tyrosine-specific protein kinases. Moreover, it was also suggested that tyrosine kinase participates in the receptor-mediated contraction of smooth muscle (Salvo et al, 1993). The results in the present study demonstrate that the tyrosine kinase plays a role in 5-HT-induced vasocontraction, since contractile response curves to 5-HT significantly shifted by genistein.

One may argue that extracellular calcium may also participate in the contraction of vascular smooth muscle to 5-HT. However, several reports have indicated that 5-HT exerts its actions in various cell systems via activation of phospholipase C which leads to the hydrolysis of membrane phosphoinositides, particularly phosphatidylinositol 4, 5-bisphosphate (Conn & Sanders-Bush et al, 1985; Litosch et al, 1985; Nakaki et al, 1985). In addition, Karaki et al (1979) have found that the phasic contractile responses to 5-HT are due to a release of intracellular calcium, since they are not blocked by agents such as verapamil and lanthanum that decrease the transmembrane movement of calcium. It has also been observed that KCl-induced contraction, which is not coupled to phosphoinositide hydorlysis, is not associated with an increase in phosphoinositide metabolism (Turla & Webb, 1990). All these findings support the hypothesis that an increase in phosphoinositide metabolism after 5-HT stimulation is not simply a nonspecific vasoconstrictor effect. Therefore, the attenuated contractile responsiveness to 5-HT-induced by genistein may be explained by an altered activity of tyrosine-specific protein kinases, suggesting that the 5-HT-induced contraction is mediated, at least in part, by an activation of tyrosine kinases.

The magnitude of the shift in the concentration-response curves by genistein was similar in DOCA-salt hypertensive and normotensive arteries. Interestingly, despite the similar sensitivity to the tyrosine kinase inhibitor, an isometric tension development induced by 5-HT was different between DOCA-salt hypertensive and control rats. The force generated in response to 5-HT was less affected by genistein in carotid strips from DOCA-salt hypertensive rats than in those from the control, suggesting that the role of tyrosine kinases may be altered in hypertensive rats. It has been known that hypertrophy, hyperplasia and polyploidy of smooth muscle cells are important features of the vasculature from hypertensive animals (Owens et al, 1981). In an examination of the cascade of events leading to cell growth, Paquet et al (1989) have observed that the proliferating activity of cultured smooth muscle cells from spontaneously hypertensive rats is more enhanced than those from normotensive rats. Hyperplasia and hypertrophy, which can be induced by experimental hypertension, may be related to the increase in blood pressure. However, the results in the present study suggest that the role of tyrosine kinases in vascular contractility is reduced in hypertension, since the effects of genistein are attenuated in DOCA-salt hypertensive rats. Several studies have found that vascular abnormalities in hypertension may be the result of alterations in the phosphoinositide signalling system (Hagerty & Ollerenshaw, 1987; Vehara et al, 1988; Turla & Webb, 1990). One cannot ruled out the possibility that smooth muscle contracion induced by 5-HT might be less dependent on tyrosine kinases, enzymes which are concerned with growth factor receptors, due to an augmented phosphoinositide metabolism in hypertensive animals.

The degree of genistein-induced relaxation of the vasculature precontracted with ED_{50} of 5-HT was markedly inhibited in DOCA-salt hypertensive rats. These observations also indicate that 5-HT-induced vasoconstriction may be less dependent on tyrosine kinases in hypertension. Similar results were found by

Sauro & Thomas (1993) in aorta from hypertensive rats. They observed the aortic responses to platelet derived growth factor (PDGF) in spontaneously hypertensive and normotensive rats in the absence and presence of tyrphostin, an another inhibitor of tyrosine kinases, and found that the responsiveness to tyrphostin-induced vasorelaxation is decreased in hypertensive rats. Additionally, they have also shown that basal and PDGF-stimulated protein tyrosine kinase activities are enhanced in hypertensive rats. Although the tyrosine kinase activity levels were not measured in the present study, the tyrosine kinase activity might be altered in DOCA-salt hypertensive rats.

A number of tyrosine kinase inhibitors have been developed to provide tools to study potential biological roles of these enzymes (Akiyama et al, 1987; Yaish et al, 1988; Gazit et al, 1989). Although tyrosine kinase inhibitor may also inhibit protein kinase C (Bishop et al, 1990), it has been observed that genistein rather selectively inhibits tyrosine kinases of the c-src family, including the EGF-urogastrone receptor kinase, without affecting other protein kinases, such as kinase C, cAMP-dependent protein kinase and phosphorylase kinase (Akiyama et al, 1987). In our study, genistein did not affect the contractile responses to PDBu, a protein kinase C activator, in both DOCA-salt hypertensive and control rats. Taken together, it seems likely that genistein acts specifically on tyrosine kinases.

In summary, It is suggested that the tyrosine kinase plays a role in 5-HT-induced contraction in carotid arterial smooth muscle, which may be reduced in DOCA-salt hypertension.

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