Effects of Calmodulin Antagonist (W-7) on Phorbol Ester (PMA)-induced Contractile Response in Isolated Rat Aorta

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Abstract

The aim of this study was to investigate effects of calmodulin antagonist (W-7) on the contractile response of the rat aorta induced by activation of protein kinase C (PKC) by phorbol ester. Phorbol 12-myristate 13-acetate (PMA) produced biphasic contraction i.e., a sustained contraction (initial contraction) and 17.9 ± 1.7 min later, this progressively developed contraction was changed to a delayed contraction superimposed on the initial contraction. The delayed contraction was completely inhibited by treatment with nicardipine. The onset of the delayed contraction was significantly delayed by treatment with W-7, whereas same concentration of W-7 showed a weak relaxant effect (10%) on the PMA-induced maximal contraction of aorta. Higher concentration of W-7 strongly inhibited PMA-induced sustained contraction. These results suggest that PMA-induced biphasic contractile response may be regulated by calmodulin.

Key words: phorbol ester, PMA, voltage-dependent Ca²⁺ channels, calmodulin, W-7.

Introduction

Protein kinase C is known to play an important role in the contractile responses of vascular smooth muscle. Phorbol esters have been reported to stimulate protein kinase C (PKC) and are used for an exogenous activator of PKC (Nishizuka, 1984; Rasmussen *et al.*, 1987; Haller *et al.*, 1990). It has been reported that phorbol esters produce sustained contraction in several arterial tissues (Rasmussen *et al.*, 1984; Barban *et al.*, 1985; Gleason and Flaim, 1986; Sybertz *et al.*, 1986; Itoh and Lederis, 1987; Jiang and Morgan, 1987; Singer and Baker, 1987). We have recently reported that, in the helical strip of rat aorta, phorbol 12-myristate 13-acetate (PMA) produced biphasic contraction, i.e., sustained contraction followed by a delayed response superimposed on the sustained contraction, and that this delayed contraction was abolished by the removal of extracellular Ca²⁺ or treatment with nicardipine, a voltage-dependent Ca²⁺-channel blocker, whereas sustained contraction was not affected by these treatment (Suenaga *et*

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al., 1993a, b).

Activation of PKC produces contraction of the smooth muscle directory, and activated PKC by phorbol esters may be mediated through Ca^{2+} -dependent activation of myosin light chain kinase (Singer and Baker, 1987; Rembold and Murphy, 1988). It also has been reported that phorbol esters are shown to induce translocation of PKC in arteries, and the translocation is temporally associated with the contractile response (Chiu *et al.*, 1987; Walsh *et al.*, 1996; Tazi *et al.*, 2000). Recently, Chuprun *et al.* (1991) have demonstrated that calmidazolium, a calmodulin antagonist, inhibits PMA-induced translocation of PKC and attenuates PMA-induced contraction, suggesting that calmodulin may be involved in phorbol esters-induced contraction of the smooth muscle.

The aim of this study was to determine whether PMA-induced biphasic response of the rat aorta is regulated by calmodulin.

Materials and Methods

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (which is accredited by the Ministry of Education, Sciences, Sports and Culture, Japan).

Preparation of aortic tissue

Male Wistar rats (Charles River, Japan), weighing 250–350 g, were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and killed by bleeding from carotid arteries. The thoracic aorta was rapidly excised and placed into oxygenated modified Krebs-Henseleit solution (KHS) that consisted of (mM): NaCl 118; KCl 4.7; CaCl_2 1.8; NaHCO_3 25.0; MgSO_4 1.2; NaH_2PO_4 1.2 and dextrose 11.0. The thoracic aorta was cleaned of loosely adhering fat and connective tissue and cut into helical strips 2 mm in width and 20 mm length. The endothelium was removed by rubbing the intimal surface with a cotton applicator. The tissues were suspended in organ baths containing 10 ml of well-oxygenated (95% O_2 –5% CO_2) KHS at 37°C. Contractile responses were measured by using force-displacement transducer (Nihon Kohden, TB-611) and recorded on a pen recorder (Yokogawa, LR4200). The tissues were equilibrated for 60 min under a resting tension of 1.0 g. During this period, the KHS in the bath was replaced with a fresh solution at 20-min intervals. After equilibration, all aorta preparations were contracted by treatment with 10^{-7} M NE to ensure stabilization of the smooth muscles, and then 10^{-5} M acetylcholine was added to the bath. The absence of functional endothelial cells was confirmed by the lack of ACh-induced relaxation.

PMA-induced contraction

After equilibration, 3×10^{-5} M W-7 or vehicle was treated to the tissue for 30 min, and then 10^{-6} M PMA was applied to the organ bath. These contractile response was observed for 240 min. Half the number of these tissues were treated with nicardipine before 10 min of PMA application. Other aortic tissues were pre-contracted by 10^{-6} M PMA and then W-7 was

cumulatively applied.

Drugs

Calphostin C, nicardipine hydrochloride, norepinephrine hydrochloride, phorbol 12-myristate 13-acetate, staurosporine and W-7 (N-{6-aminohexyl}-5-chloro-1-naphthalene-sulfonamide) were purchased from Sigma Chemical Co. (St.Louis, MO). Acetylcholine chloride was purchased from Daiichi Pharmaceuticals (Tokyo, Japan). Calphostin C, PMA and staurosporine were dissolved in dimethylsulfoxide (DMSO). The final concentration of DMSO in the organ bath was 0.1%, and did not affect contraction or relaxation. Nicardipine was dissolved in ethanol and diluted by distilled water. Other drugs were dissolved in distilled water.

Data analysis

The results shown in the text and figures are expressed as the mean $\pm$ S.E. Statistical differences were assessed by Dunnett's multiple comparison test following a one-way analysis of variance.

Results

Typical contractions of aortic strips in response to PMA are illustrated in Fig. 1A. In the aorta, 10^{-6} M PMA produced a sustained, slowly developing contraction (initial contraction) and 17.9 ± 1.7 min (n=6) later, this progressively developed contraction was changed to a delayed response superimposed on the initial contraction. The time-course of PMA-induced contraction is summarized in Fig. 1B. These biphasic contraction was abolished by 10^{-7} M staurosporine (data not shown). Calphostin C, a specific inhibitor of PKC, also inhibited these biphasic contraction. The maximal contractions induced by PMA in the absence or presence of 3×10^{-7} M calphostin C were 1036.3 ± 31.3 mg (n=6) and 301.7 ± 35.9 mg (n=5), respectively. Moreover, the delayed contraction was abolished by pretreatment with 10^{-7} M nicardipine but sustained contraction was remained. Pattern of PMA-induced contractile response was altered by pretreatment of 3×10^{-5} M W-7. Especially, onset of PMA-induced delayed response, which was depend on external Ca^{2+} , was significantly delayed as shown in Figs. 1A&B (control, 17.9 ± 1.7 min, n=6; treated with W-7, 158.3 ± 13.9 min, n=5, $p < 0.01$).

As shown in Fig. 2, however, 3×10^{-5} M W-7 showed only 10% inhibition of the maximal contraction induced by PMA when W-7 was added during the plateau phase of the contractile response to PMA. Higher concentration of W-7 (10^{-4} M) inhibited strongly 10^{-6} M PMA-precontracted aortic strip.

Discussion

In the present study, we found that W-7, a specific inhibitor of calmodulin, delayed the onset of PMA-induced delayed response, which was depend on external Ca^{2+} , whereas PMA-induced slowly developing contraction (initial contraction) was not affected by W-7.

It has been reported that phorbol ester-induced vascular contraction was caused by an influx

Fig. 1. A: Typical recordings of 10^{-6} M phorbol 12-myristate 13-acetate (PMA)-induced contractile response in the absence (upper) or presence (lower) of 3×10^{-5} M W-7. B: Time-dependent contractile response to 10^{-6} M PMA of rat aorta. Aortic strips were treated with vehicle (○), 10^{-7} M nicardipine (□), 3×10^{-5} M W-7 (●) or 10^{-7} M nicardipine and 3×10^{-5} M W-7 (△). Each point represents the mean $\pm$ S.E. of 5 to 6 animals.

of external Ca^{2+} (Rasmussen *et al.*, 1984; Baraban *et al.*, 1985; Fish and Sperti, 1988), and others reported that contractions induced by phorbol 12, 13-dibutyrate in the rabbit aorta (Sybertz *et al.*, 1986) or 12-O-tetradecanoylphorbol-13-acetate in the rat aorta (Spedding, 1987) were not sensitive to the absence of calcium or to Ca^{2+} entry blockers. These discrepancies are difficult to explain, but may be, at least in part, attributed to differences in the animals used or in the methods of tissue preparation. Indeed, we also reported that in the isolated rat aorta, phorbol 12-myristate 13-acetate (PMA) induced a biphasic contraction, i.e., a sustained contraction followed by a delayed response superimposed on the sustained contraction, and that this delayed contraction was abolished by the removal of extracellular Ca^{2+} or treatment with nicardipine, a voltage-dependent Ca^{2+} -channel blocker (Suenaga *et al.*, 1993a, b). Furthermore, we reported that the activation of voltage-dependent Ca^{2+} -channels by PMA is mediated by islet-activating protein (IAP)-sensitive GTP-binding protein (Suenaga *et al.*, 1996). PMA are known to cause irreversible translocation of PKC (Gopalakrishna *et al.*, 1986). Thus, it may be speculate the PMA-induced contraction as follows. PMA activates PKC in the aortic smooth muscle; an activated PKC translocates to the membrane; the translocated and activated PKC may increase Ca^{2+} sensitivity of myosin light chain phosphorylation in aortic smooth muscle and cause a sustained contraction; the translocated PKC also activates IAP-sensitive GTP-binding protein;

Fig. 2. Effect of W-7 on phorbol 12-myristate 13-acetate (PMA)-induced contraction of rat aorta. Aortic strips were precontracted with 10^{-6} M PMA and then W-7 was applied cumulatively. Results were expressed as percentage of decreased tension of contractile force induced by PMA and mean $\pm$ S.E. of 5 animals.

the activated GTP-binding protein may activate voltage-dependent Ca^{2+} -channels, thereby resulting in Ca^{2+} -dependent delayed contraction.

In the present study, onset of a delayed contraction in response to PMA was delayed by pretreatment of the aorta with W-7. Recently, Chuprun *et al.* (1991) have reported that calmodulin antagonist inhibits the development of PMA-induced contraction through inhibition of PKC translocation, suggesting involvement of calmodulin in the PMA-induced contraction. It is likely, therefore, that impairment of PKC translocation by W-7 may delay the onset of delayed contraction. It was presently demonstrated that the W-7 greatly delayed the onset of PMA-induced delayed contraction, whereas the drug was relatively weak relaxants when added during the plateau phase of the contractile response to PMA. This result also strongly suggest that W-7 may inhibit translocation of PKC and then may delay the onset of delayed contraction by prior exposure of W-7. Since sustained contraction also inhibited by higher concentration of W-7, calmodulin may be involved in PMA-induced biphasic contraction.

In conclusion, PMA produced biphasic contraction, i.e., a sustained contraction followed by a delayed response superimposed on the sustained contraction. Calmodulin antagonist, W-7, delayed the onset of delayed contraction, whereas W-7 was relatively weak relaxants when added during the plateau phase of the contractile response to PMA. These results strongly suggest that PMA-induced contractile response of rat aorta may be, at least in part, due to activation of calmodulin.

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