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Effect of Cyclic Nucleotides on Phorbol Ester-Induced Contraction in Rabbit Carotid Artery

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=ABSTRACT=

This study was designed to clarify the action of cyclic nucleotides, cyclic AMP and cyclic GMP, on phorbol 12,13-dibutyrate (PDBu)-induced contraction in rings isolated from rabbit carotid artery. Arterial rings, 2 mm in width, were myographied isometrically in an isolated organ bath. PDBu produced slowly developing, sustained contraction in rabbit carotid artery, in a dose dependent manner, which was independent of extracellular Ca^{2+}. PDBu-induced contraction was relaxed by staurosporine, which suggests that PDBu-induced contraction is mediated by protein kinase C (PKC). $^{45}Ca^{2+}$ uptake by rabbit carotid artery was increased by PDBu during depolarization, but not in control. Isoproterenol and sodium nitroprusside (SNP) relaxed phenylephrine-induced contraction. However, SNP but not isoproterenol relaxed the contraction induced by PDBu. Acetylcholine relaxed PDBu-induced contraction in the presence of the endothelium. 8-bromo-cyclic AMP, a permeable analogue of cyclic AMP, suppressed phenylephrine-induced contraction but not PDBu-induced contraction. 8-bromo cyclic GMP relaxed both of them with dose dependency. A large dose of forskolin relaxed PDBu-induced contraction. PDBu increased cyclic AMP without considerable change in the level of cyclic GMP.

Based on these findings, PDBu-induced contraction of rabbit carotid artery was relaxed by cyclic GMP more effectively than cyclic AMP, and the action of cyclic AMP could be mediated by cyclic GMP dependent protein kinase. Therefore it is suggested that the antagonistic action between protein kinase C and cyclic GMP-dependent protein kinase plays a major role in the regulation of vascular tone.

Key Words: Phorbol ester, Protein kinase C, Cyclic nucleotides, Vascular smooth muscle

INTRODUCTION

In smooth muscle, Ca^{2+} plays a regulatory role in contraction (Bolzer, 1969; Bolton, 1973; Somlyo et al, 1982), and it was assumed that a mechanism similar to the Ca^{2+}-activated actin-myosin cross bridge mechanism of skeletal muscle also operates in smooth muscle.

The contraction of smooth muscle consists of an initial phase in which the concentration of free Ca^{2+} is increased, producing myosin light chain (MLC) phosphorylation by the calmodulin pathway, and a late phase of tension maintenance that is associated with a reduction in cytosolic Ca^{2+}, a

decrease in the MLC phosphorylation level, and oxygen consumption slightly higher than basal state (Sommerville and Hartshorne, 1986; Rasmussen et al, 1987). Concerning the late phase, a sustained contraction of the smooth muscle, there are two hypotheses, latch bridge theory (Dillon et al, 1981) and phosphorylation of the filamin-actin-desmin fibrillar domain theory (Rasmussen et al, 1987). Rasmussen et al (1987) reported that protein kinase C (PKC) activation, which phosphorylates intermediate filament protein, is responsible for this sustained contraction in smooth muscle.

Exposure of smooth muscle strips to phorbol ester leads to a slowly developing contraction which lasts for several hours (Forder et al, 1983; Danthuluri & Deth, 1984; Park & Rasmussen, 1985). It has been shown that phorbol esters induce a sustained contraction in chemically skinned vascular smooth muscle when the Ca^{2+} concentration is maintained at 100 nM (Chatterjee & Tejada, 1986). Agonist-induced increase in diacylglycerol (DAG) concentration is sustained (Griendling et al, 1986; Takuwa et al, 1986; Takuwa et al, 1988), but the increase in cytosolic free Ca^{2+} concentration is only transient (Morgan & Morgan, 1984; Alexander et al, 1985). Therefore it was suggested that pharmacological activation of PKC could induce a slowly developing contractile response in vascular smooth muscle.

Cyclic AMP dependent protein kinase and cyclic GMP dependent protein kinase modulate the cellular function through phosphorylation of substrate proteins (Edelman et al, 1987), and relax smooth muscle (Kamm & Stull, 1985). Elevations in cyclic nucleotide concentration may relax smooth muscle by several different mechanisms; decreasing $[Ca^{2+}]_i$, decreasing the Ca^{2+} sensitivity of MLC phosphorylation, or uncoupling force from MLC phosphorylation (Rembold, 1992).

The interaction between cyclic nucleotides and PKC in smooth muscle remains uncovered.

A sustained contraction of vascular smooth muscle may play a role in the pathogenesis of hypertension. Thus phorbol ester-induced sustained contraction of vascular smooth muscle is a focus for many investigators nowadays.

The aim of the present study was to investigate the action of PKC and cyclic nucleotides, and interaction between them in regulation of vascular tone. To clarify the characteristics of vascular contraction induced by the activation of PKC, the followings were studied: extracellular Ca^{2+} dependency, Ca^{2+} flux, effects of various vasorelaxants, and effects of cyclic nucleotides on the phorbol 12,13-dibutyrate (PDBu)-induced contraction of isolated rabbit carotid artery. The present studies demonstrate that PDBu-induced contraction was more effectively antagonized by intracellular cyclic GMP than cyclic AMP, suggesting that the relative activities of protein kinase C and cyclic GMP dependent protein kinase play very important roles in the regulation of vascular tone.

METHODS

Tissue preparation

New Zealand White rabbits of either sex (1.5~2.5 kg) were sacrificed by a blow to the neck. Subsequently both carotid arteries were isolated carefully and placed in a petri dish containing Krebs-Henseleit solution (KHS) of the following composition (mM): NaCl 115, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, dextrose 10. In this solution gased with a mixture of 95% O_2-5% CO_2, arterial rings, 2 mm in width, were prepared after removing adipose and connective tissue.

Measurement of smooth muscle contraction

The rings were suspended vertically under 1 g tension in KHS with two L shaped stainless steel

pins. The bath solution was maintained at 37°C and gased with a mixture of 95% O_2-5% CO_2. The vascular rings were allowed to equilibrate for 90 min prior to starting experiments. During the equilibration period the bath solution was replaced every 30 min. Changes in muscle tension were recorded isometrically through a force-displacement transducer (FT-03) on a Grass polygraph. The Ca^{2+}-free solution was made by adding 1 mM EGTA instead of 2.5 mM $CaCl_2$. A high K^+ solution was obtained by replacing Na^+ with an equivalent amount of K^+.

Measurement of $^{45}Ca^{2+}$ uptake

The $^{45}Ca^{2+}$ uptake by carotid arterial strips were measured at 37°C according to the methods described by Meisheri et al(1980). After 90min stabilization in the Na-HEPES buffer, tissues were incubated for 5 min in a medium containing $^{45}Ca^{2+}$ (1 μCi/ml) in the presence or absence of PDBu. The Na-HEPES buffer contained (mM) NaCl 120, KCl 5.0, $CaCl_2$ 2.0, $MgCl_2$ 1.2, glucose 10 and HEPES 10 (pH 7.4 with Tris). Upon completion of the incubation, tissues were placed in 100 ml ice-cold La-HEPES buffer for 5 sec, and then placed in 4 ml of the same buffer for 45 min in order to remove the extracellularly bound $^{45}Ca^{2+}$. The La-HEPES buffer contained (mM) NaCl 120, KCl 5.0, $MgCl_2$ 1.2, $LaCl_3$ 10, glucose 10 and HEPES 10 (pH 7.4 with Tris). The tissues were then blotted, weighed and incubated overnight in 200 μl of perchloric acid-H_2O_2 mixture (1:1). After the tissue was dissolved, the radioactivity of $^{45}Ca^{2+}$ was counted in a liquid scintillation counter (Packard Tricarb 300C). The amount of net $^{45}Ca^{2+}$ uptake was expressed as μmole/kg/5 min.

Measurement of cyclic AMP and cyclic GMP formation

Smooth muscle tissues excised from rabbit carotid arteries were prepared in cold KHS. Dissected tissues of 10 mg wet weight were kept at 37°C for 60 min in KHS as the control. PDBu and other agents were added to the media 15 min before the end of the 60 min incubation period. After 60 min incubation, the tissue was frozen with CO_2 cryo and homogenized in 6% trichloroacetic acid. It was then centrifuged for 25 min at 2000 $\times$ g. The level of cyclic AMP and cyclic GMP in extracts were measured using a radioimmunoassay kit obtained from Amersham (Vesin and Harbon, 1974). The centrifuged pellets were dissolved in 0.3% deoxycholic acid solution for protein determination (Bradford, 1976). Cyclic AMP and cyclic GMP levels were expressed as pmol/mg protein/15min.

Drugs and chemicals

The Drugs, phenylephrine, phorbol 12, 13-dibutyrate(PDBu), sodium nitroprusside (SNP), isoproterenol, acetylcholine, staurosporine, nifedipine and EGTA (ethyleneglycol-bis(β-aminoethylether)-N-N'-tetra-acetic acid) were purchased from Sigma Chemical Co. 8-bromo-cyclic AMP and 8-bromo-cyclic GMP were purchased from RBI. All other chemicals were of analytical grade. Stock solutions of PDBu and staurosporine were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the bath solution was not less than 0.2% and at this concentration of DMSO caused only a slight decrease of tension.

Statistical analysis

Results were expressed as the mean ± SE. The difference between two mean values was analysed by Student's t-test for unpaired observations. The difference was considered statistically significant when $P < 0.05$.

RESULTS

Contractile responses induced by PDBu

PDBu (0.1 μM) in KHS produced a slow and progressive increase in tension of the carotid arterial rings (Fig. 1 A). The contraction reached a plateau after 15~45 min. The dose response relationship of PDBu-induced contraction is presented in Fig. 1 B. The threshold dose of PDBu to evoke a contractile response was 10 nM, and the ED_{50} was about 0.1 μM. The PDBu-induced contraction was not affected by tetrodotoxin (1 μM) or phentolamine (1 μM) pretreatment (data not shown). PDBu-induced contraction was reproducible, but in order to return to basal tone prolonged washing for about 2 hours was required (data not shown). Replacement of the buffer with a Ca^{2+}-free KHS containing 1 mM EGTA in the presence of 0.1 μM PDBu did not reduce PDBu-induced contraction (Fig. 2).

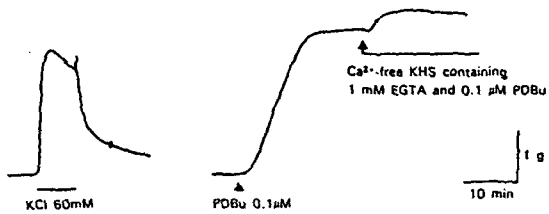

Fig. 2. Effect of extracellular Ca^{2+} on PDBu-induced contraction. PDBu-induced contraction was not altered by replacing bathing medium with Ca^{2+}-free KHS containing 1 mM EGTA and 0.1 μM PDBu.

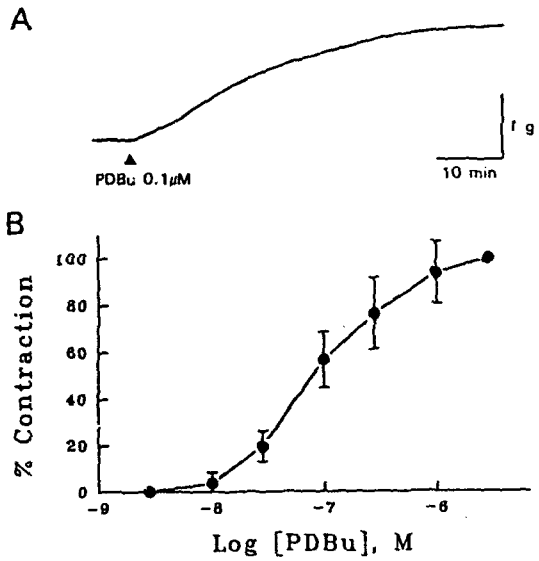

Fig. 1. A: Typical tracing of phorbol 12,13-dibutyrate (PDBu)-induced contraction in the rabbit carotid artery in KHS. B: Dose-response curve of PDBu-induced contraction. The magnitude of contraction is presented as % of maximum contraction induced by 3 × 10^{-6} M PDBu. Vertical bars indicate SE (n=5).

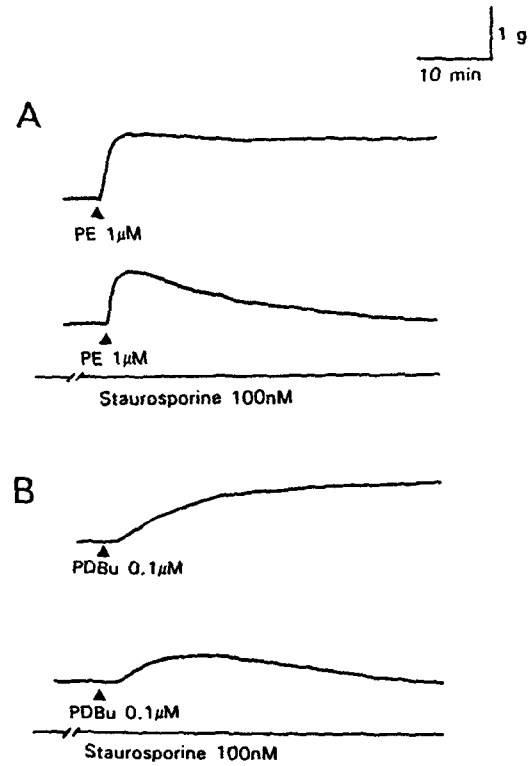

Fig. 3. The effect of staurosporine on the phenylephrine (A) and PDBu (B) -induced contractions.

Effect of PKC inhibitors on PDBu-induced responses

Staurosporine is a potent inhibitor of PKC. Pretreatment with staurosporine (100 nM) inhibited phenylephrine-induced contraction, but the phasic component was not influenced and only tonic component was reduced (Fig. 3A). PDBu-induced contraction was transiently produced with staurosporine pretreatment (Fig. 3B).

Effect of PDBu on $^{45}Ca^{2+}$ flux

PDBu did not change $^{45}Ca^{2+}$ uptake for 5 min in normal KHS (Fig. 4). But under depolarization with a KCl (120 mM) solution, PDBu stimulated $^{45}Ca^{2+}$ uptake.

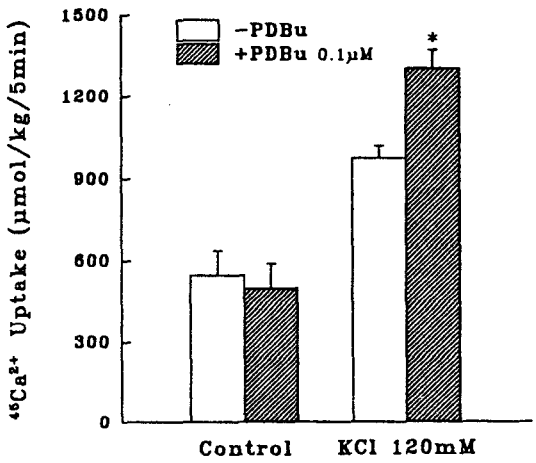

*Fig. 4. Effect of PDBu (0.1 μM) on $^{45}Ca^{2+}$ uptake by intact rabbit carotid artery. PDBu was added 10 min prior to the measurement of $^{45}Ca^{2+}$ uptake in control tissues. $^{45}Ca^{2+}$ was added 10 min after exposure of tissue to Na-HEPES buffer containing 120 mM KCl in the presence or absence of PDBu. Data are expressed as means ± SE (n=5). *, P < 0.05, compared with the uptake in the absence of PDBu.*

Effects of cyclic nucleotides and its agonists on PDBu-induced contraction

Fig. 5 depicts the effects of isoproterenol, an adenylate cyclase activator, and nitroprusside, a guanylate cyclase activator, on phenylephrine- and PDBu-induced contraction. Phenylephrine-induced contraction was relaxed by isoproterenol and SNP. PDBu-induced contraction was also relaxed by SNP but not by isoproterenol.

Acetylcholine can modulate vascular tone by endothelium dependent relaxing factor which acti-

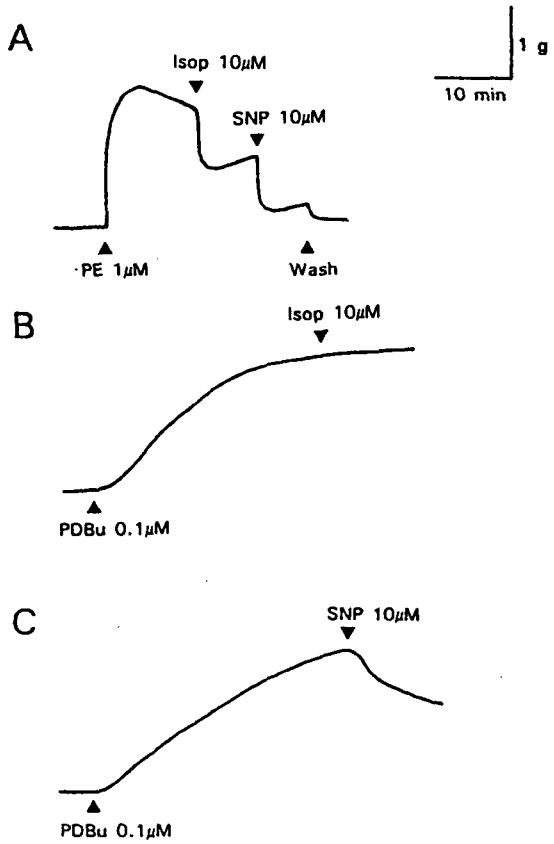

Fig. 5. Effects of isoproterenol and nitroprusside on phenylephrine (A) and PDBu (B)-induced contraction. PE, phenylephrine; Isop, isoproterenol; SNP, sodium nitroprusside.

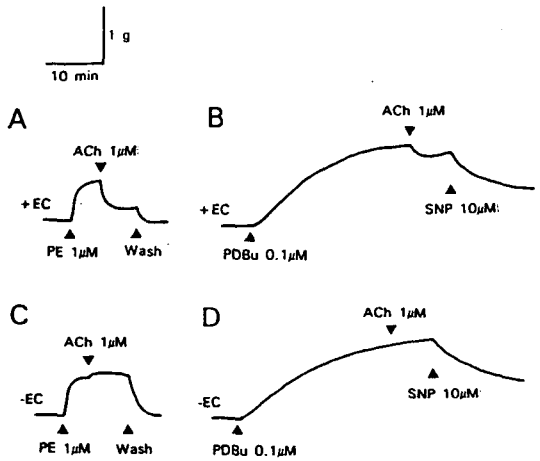

Fig. 6. Endothelium dependent relaxation induced by acetylcholine in vascular ring precontracted with phenylephrine and PDBu. +EC, intact endothelium; −EC, denuded endothelium; ACh, acetylcholine; SNP, sodium nitroprusside.

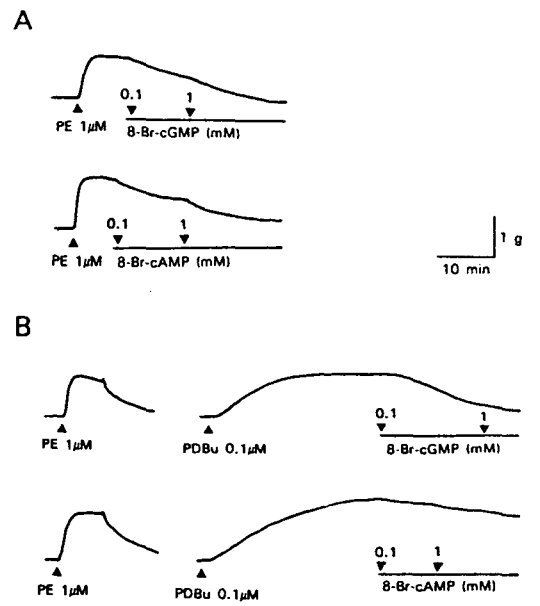

Fig. 7. Effects of permeable cyclic nucleotides on phenylephrine (A) and PDBu (B)-induced contraction. PE, phenylephrine.

vates soluble guanylate cyclase (Furchgott & Zawadzki, 1980). Phenylephrine- and PDBu-induced contractions were relaxed by acetylcholine in the presence of intact endothelium (Fig. 6 A and B). Vascular rings denuded of endothelium were not relaxed by acetylcholine (Fig. 6 C and D). This means that the effect of acetylcholine on the PDBu-induced response was dependent on endothelium.

In rabbit carotid artery, PDBu-induced contraction was relaxed by acetylcholine but not by isoproterenol. This finding suggests that intracellular cyclic GMP but not cyclic AMP has relaxing effect on PDBu-induced contraction. In order to characterize this effect further, the effect of permeable analogue of cyclic nucleotides such as 8-bromo-cyclic AMP and 8-bromo-cyclic GMP, were examined in phenylephrine and PDBu-induced contraction. As shown in Fig. 7A, phenylephrine-induced contraction was relaxed by 8-bromo-cyclic AMP and 8-bromo-cyclic GMP, but PDBu induced contraction was relaxed by 8-bromo-cyclic GMP only (Fig. 7B).

Forskolin is a novel activator of the catalytic subunit of adenylate cyclase and is now widely used to delineate the role of cyclic AMP in

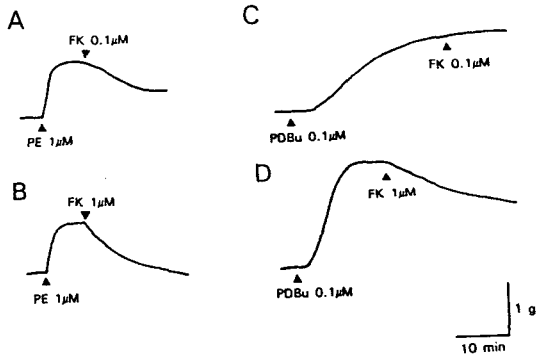

Fig. 8. Effect of forskolin on phenylephrine (A,B) and PDBu (C,D)-induced contraction. FK, forskolin.

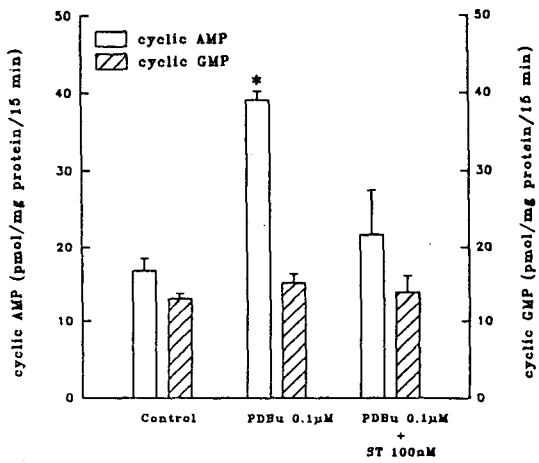

*Fig. 9. Effect of PDBu on tissue cyclic nucleotide production. Data are expressed as means ± SE (n=6). *, P < 0.05, compared with control. ST, staurosporine.*

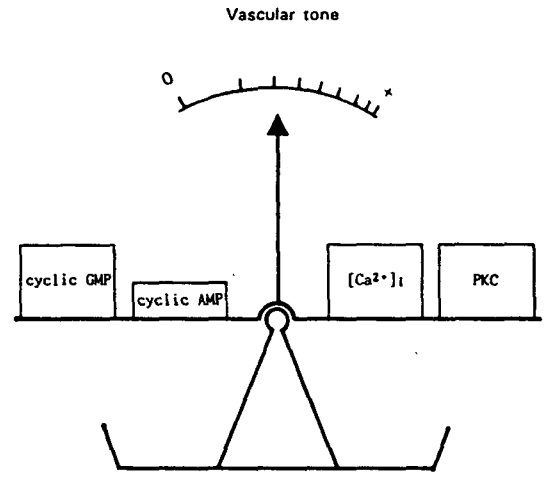

Fig. 10. Hypothetical model for the regulation of contractile status of vascular smooth muscle. The balance position of the arrow is determined by the difference between the weight value of cyclic nucleotides and PKC plus $[Ca^{2+}]_i$. In the resting state, there is some contractility, determined by the balance between the right and left. The action of an agonist which increases intracellular Ca^{2+} level and/or PKC activation, is also determined by the activity of the left as counterpart. The larger area of cyclic GMP than of cyclic AMP means that the former has a more dominant effect on the relaxation of vascular smooth muscle. In the case of a decrease in cyclic nucleotide and an increase in $[Ca^{2+}]_i$ or PKC, vascular smooth muscle contracts to a level higher than the basal tone. Conversely, an increase in cyclic nucleotide and a decrease in $[Ca^{2+}]_i$ or PKC will lead to vasorelaxation. PKC, the activity of protein kinase C; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration.

modulating cellular functions (Seamon & Daly, 1983). It relaxed phenylephrine- and PDBu-induced contraction (Fig. 8), but there was a dose related discrepancy between phenylephrine- and PDBu-induced contraction. Although 1 μM of forskolin relaxed all of the responses induced by phenylephrine and PDBu, 0.1 μM of forskolin relaxed only phenylephrine-induced contraction, and did not affect the contraction induced by 0.1 μM PDBu (Fig. 8C).

Effect of PDBu on the production of cyclic nucleotides

Fig. 9 shows the effect of PDBu on the production of cyclic AMP and cyclic GMP in the rabbit carotid artery during 15 min incubation. In comparision to control, PDBu increased the amount of cyclic AMP about two-fold without considerable change in the production of cyclic GMP. The effect of PDBu on cyclic AMP production was prevented by application of 100 nM of staurosporine.

DISCUSSION

PKC activation by DAG has been proposed to maintain smooth muscle contraction by agonist stimulation (Takuwa et al, 1986; Rasmussen et al, 1987). Optimal activation of PKC requires free Ca^{2+}, membrane phospholipid (especially phosphatidylserine), and DAG (Howe & Abdel-Latif, 1988). Active phorbol ester, PDBu, mimics the

action of DAG to stimulate PKC (Castagna et al, 1982; Nishizuka, 1984).

The contraction of smooth muscle in response to contractile agonists is mediated by an increase in the concentration of intracellular free Ca^{2+}, which declines to near basal levels after a few minutes although contraction is sustained (Sasaki et al, 1991). In intact tracheal (Takuwa et al, 1986) and carotid artery (Takuwa et al, 1988) smooth muscle, DAG levels in the cell remain elevated after agonist-stimulation, implying a role of PKC in the maintenance of tone. Phorbol esters are well known to produce slowly developing and sustained contraction in various vascular smooth muscle preparations by activation of PKC (Rasmussen et al, 1987).

In the present findings, exposure of the rabbit carotid artery to PDBu in normal KHS produced slowly developing and sustained contractile responses (Fig. 1) which were not influenced by the removal of extracellular Ca^{2+} (Fig. 2). This result suggests that PDBu-induced sustained contraction is independent of extracellualar Ca^{2+}. A contributory role of protein kinase C activation is supported by the observation that staurosporine suppressed contraction induced by PDBu in carotid artery (Fig. 3).

Though the adventitial layer of vascular strips may retain sufficent quantities of extracellular Ca^{2+} (Karaki et al, 1979), excess EGTA did not show any significant effect on PDBu-induced contraction (Fig. 2). The plateau response to PDBu was not rapidly reversed by repeated washings (data not shown), and this observation suggests that the contractile responses of rabbit carotid artery to PDBu are due to an action of the compound at an intracellular site. Replacement of the bath solution with Ca^{2+}-free solution containing 1mM EGTA produced a slight increase in tension (Fig. 2A). This may be due to EGTA, a high concentration of which tends to impair cellular function by increasing membrane permeability to small ions (Winegrad, 1971) or inducing cellular swelling (MaCknight & Leaf, 1977). The presence or absence of endothelial cells did not change the PDBu-induced response (Fig. 6), indicating a direct effect of this agent on vascular smooth muscle. Abraham and Rice (1992) reported that PDBu-induced contractile responses in vascular smooth muscle varied from none to small and transient, and the reason for this may be that the concentration of intracellular calcium is at the threshold for PKC activation. However, Collins et al (1992) reported that phenylephrine, an α_1-agonist, could induce contraction of vascular smooth muscle at constant intracellular free Ca^{2+} concentration, and constant agonist response was not influenced by a change of pCa between 8.7 and 7.0. This may be an evidence for the existence of Ca-independent isozyme of PKC.

Staurosporine (Tamaoki et al, 1986) is a relatively specific PKC inhibitor. In the present study, PDBu-induced contraction was reduced by staurosporine (Fig. 3). This suggests that PDBu-induced contraction was due to PKC activation.

PDBu increased Ca^{2+} uptake in depolarized rabbit carotid artery strip but not in control (Fig. 4). This means that PDBu increases Ca^{2+} flux via the inactivated voltage dependent Ca^{2+} channel without any change in membrane permeability. Without concomitant change in membrane potential, PDBu does not increase intracellular Ca^{2+} concentration.

Isoproterenol, a β-adrenergic agonist, is known to activate adenylate cyclase, and to relax vascular smooth muscle by increasing the level of cyclic AMP. In this study PDBu-induced contraction did not respond to isoproterenol, though phenylephrine-induced contraction was relaxed by isoproterenol (Fig. 5).

Acetylcholine partially suppressed PDBu-induced contraction in rabbit carotid artery with

intact endothelium (Fig. 6). After removal of endothelium, the effect disappeared. Thus an endothelium dependent relaxing factor, nitric oxide, mediates the action of acetylcholine on PDBu-induced contraction. Nitric oxide exerts its action by stimulating soluble guanylate cyclase in smooth muscle cells (Rapaport & Murad, 1983; Ignarro, 1989). The vasorelaxation by cyclic GMP is believed to decrease the intracellular Ca^{2+} level (Rembold, 1992). In this study, however, PDBu-induced contraction was relaxed by sodium nitroprusside (Fig. 6). The inhibition of PDBu-induced contraction by cyclic GMP may be due to a decrease in the Ca^{2+}-sensitivity of MLC phosphorylation (Karaki et al, 1988) or to an uncoupling of force from myosin phosphorylation (MacDaniel et al, 1992).

Nishimura and Van Breemen (1989) reported that PDBu-induced contraction was relaxed by cyclic AMP and cyclic GMP with reduced Ca^{2+}-sensitivity of contractile apparatus in α-toxin-permeabilized rat mesentric artery. Although this study was not conducted in permeabilized vascular smooth muscle, PDBu-induced contraction was not affected by 8-bromo-cyclic AMP up to 1mM (Fig. 7). The discrepancy between the permeabilized condition and intact tissue may be due to tissue specificity or to other actions of permeable cyclic nucleotides.

Forskolin, known to activate adenylate cyclase directly, was effective at 1 μM on PDBu-induced contraction. At 0.1 μM forskolin was effective only on phenylephrine-induced contractions (Fig. 8). This is consistent with the report of Rembold (1992) that high doses of forskolin relaxed phorbol ester-induced contraction in vascular smooth muscle. This finding does not support the idea that cyclic AMP-dependent protein kinase is involved in the inhibition of PDBu-induced contraction. Because Jiang et al (1992) reported that moderate elevation of either cyclic GMP or cyclic AMP activates intracellular cyclic GMP dependent protein kinase, thus producing relaxation of vascular smooth muscle. Lincoln et al (1990) reported that intracellular Ca^{2+} level was not decreased by cyclic AMP in a smooth muscle cell line lacking the cyclic GMP-dependent protein kinase, unless cyclic GMP-dependent protein kinase was reintroduced by electrophoresis. Cyclic GMP-dependent protein kinase can be activated by cyclic AMP, although it requires nearly a 10-fold higher cyclic AMP concentration than cyclic GMP concentration for its activation (Foster et al, 1981).

This study has found that the effect of forskolin on PDBu-induced contraction may be due to activation of cyclic GMP-dependent protein kinase stimulated by cyclic AMP.

In this study, PDBu increased tissue cyclic AMP production without any change of cyclic GMP (Fig. 9). The interaction between PKC and cyclic AMP in various tissues has been reported (Houslay, 1991). PKC avtivation induces an increase or a decrease of cyclic AMP production in different tissues (Abdel-Latif, 1991). In smooth muscle, augmentation of isoproterenol-stimulated adenylate cyclase by phorbol esters has shown in vascular smooth muscle cells (Nabika et al, 1985). In this result, PDBu increased cyclic AMP in unstimulated vascular smooth muscle, and the increase was prevented by staurosporine. This demonstrates that PKC activation could increase intracellular cyclic AMP production under physiological conditions, and represents an example of cross-interaction between PKC and cyclic AMP as second messenger systems in vascular smooth muscle. In this study, isoproterenol and 8-bromo-cyclic AMP did not affect PDBu-induced contraction, which can be partially explained by the assumption that the former increase in intracellular cyclic AMP by PDBu abbreviated the effect of any additional increase of cyclic AMP.

Haller et al (1990) reported that in resting calf

carotid artery, the amount of PKC activity associated with the membrane fraction was consistently in the range of 20~30%. This suggests that there is PKC activity associated with the plasma membrane of relaxed smooth muscle cells.

In this study, it was found that increases in intracellular Ca^{2+} or PKC activity could induce vascular contraction, which could be antagonized by an increase in intracellular cyclic nucleotide. Thus, to organize these results, the hypothetical model is presented in Fig. 10. The vasoconstrictive action of the right part and the vasorelaxant action of the left part are equal in the resting state. In the equilibrated resting state, the vascular smooth muscle has some contractility, and the basal activity of cyclic nucleotide, $[Ca^{2+}]_i$ and PKC are functioning at low levels.

If even one of the four parts is changed in vascular smooth muscle in an equilibrium relaxed or contracted state, then vascular tone is changed to a new equilibrium. In vascular smooth muscle, an increase in cyclic AMP level could give relaxattion by activation of cyclic GMP-dependent protein kinase, and an elevated intracellular Ca^{2+} level and PKC activation could synergistically contribute to the contraction. Thus each part of right and left is interacting each other part. Further quantitative and qualitative characteristics of their interaction remains to be elucidated to explain the exact control mechanism of vascular tone.

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