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Inhibitory effect of phorbol 12,13-dibutyrate on norepinephrine-induced contraction in rabbit iris dilator muscle

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Abstract

The hypothesis that the increase in Ca^{2+} sensitivity on norepinephrine-induced contraction of smooth muscles and also the decrease of the norepinephrine-induced sustained level of intracellular Ca^{2+} concentration are produced by the activation of protein kinase C was tested. Phorbol 12,13-dibutyrate (PDB; 10^{-6} M) relaxed the norepinephrine-induced sustained contraction in a concentration-dependent manner. On pretreatment with PDB a transient contraction was produced by the application of norepinephrine, but the sustained contraction was significantly reduced. The sustained elevations of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and the contraction induced by norepinephrine in fura-2-loaded preparations were decreased by the application of PDB. These inhibitory effects were antagonized by potent protein kinase inhibitors, 2-(1-(3-dimethylaminopropyl)-indol-3-yl)-3-(-indol-3-yl)-maleimide (GF 109203X) (10^{-6} M) and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7) (10^{-6} M), but were not affected by a protein kinase A/G inhibitor, *N*-(2-cinnamylaminoethyl)-5-isoquinolinesulfonamide (H-88) (10^{-6} M). The slope of the regression line for norepinephrine for $[Ca^{2+}]_i$ and tension was significantly steeper than those obtained with high K^+. Also, on pretreatment with PDB the Ca^{2+} sensitivity of the K^+-induced contraction was decreased, but the Ca^{2+} sensitivity of norepinephrine-induced contraction tended to be increased. These observations indicate that PDB induces a decrease of $[Ca^{2+}]_i$ on Ca^{2+} mobility and an increase of Ca^{2+} sensitivity on contraction of smooth muscle through the activation of protein kinase C.

Keywords: Iris dilator, rabbit; Phorbol 12,13-dibutyrate (PDB); Ca^{2+} mobilization; Protein kinase C; GF 109203X (2-(1-(3-dimethylaminopropyl)-indol-3-yl)-3-(-indol-3-yl)-maleimide); H-7 (1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride); H-88 (*N*-(2-cinnamylaminoethyl)-5-isoquinolinesulfonamide)

1. Introduction

The receptors mediating contraction and relaxation of smooth muscle respond to some intracellular transduction substances. Except in the case of Ca^{2+}-independent contraction of smooth muscle (Sakai and Uchida, 1980), Ca^{2+} has been believed to play an essential role in eliciting smooth muscle contraction and relaxation. The rise in intracellular Ca^{2+} ($[Ca^{2+}]_i$) results from at least two mechanisms: (1) mobilization of extracellular Ca^{2+} through Ca^{2+} channels; and (2) activation of intracellular Ca^{2+} release. The manner of regulation of Ca^{2+} movement during the contraction of iris smooth muscle induced by α_1-adrenoceptor stimulant has not yet been studied in detail, though α_1-adrenoceptor have been pharmacologically characterizatied in various tissues (Gross et al., 1989; Han et al., 1987; Konno and Takayanagi, 1987; Satoh et al., 1992a,b; Takayanagi et al., 1991; Takayanagi and Moriya, 1991). Han et al. (1987) and Tsujimoto et al. (1989) suggested that α_{1B}-adrenoceptor subtypes are linked to inositol phosphate formation related to the release of Ca^{2+} from Ca^{2+} stores, and that α_{1A}-adrenoceptor subtypes control the influx of extracellular Ca^{2+} via Ca^{2+} channels in vascular smooth muscle. Takayanagi et al. (1992) reported that smooth muscle of rabbit iris dilator is composed mainly of α_{1B}-adrenoceptors, and Kokubu et al. (1993) suggested that norepinephrine-induced contraction is due to an increase in the release of intracellularly sequestered Ca^{2+}. We proposed a hypothesis that, in the regulation of intracellular Ca^{2+} concentration, protein kinase C decreases phosphatidyl inositol 4,5-bisphosphate (PIP_2) breakdown (inositol 1,4,5-trisphosphate (IP_3) production) by the inhibition of phospholipase C, and simultaneously increases the Ca^{2+} sensitivity of contractile elements in muscle contraction.

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In the present experiments, we studied the effect of the protein kinase activating agent, PDB, on norepinephrine-induced contraction, and also the physiological roles of protein kinase C in changes of intracellular Ca^{2+} concentration, using fura-2-loaded and non-loaded rabbit iris dilator strips.

2. Materials and methods

2.1. Measurement of mechanical response

The dilator muscle strips were dissected from male albino rabbits weighing 2–3 kg according to method of Takayanagi and Kaneko (1982). The strips were suspended in 20-ml organ baths filled with a physiological saline solution (PSS) which contained (in mM): NaCl, 118; $MgCl_2$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 5.0 and glucose, 11.0 dissolved in distilled water. The solution contained propranolol (10^{-6} M) and yohimbine (3×10^{-7} M) to block β- and α_2-adrenoceptors, desmethylimipramine (10^{-7} M) and normetanephrine (10^{-6} M) to inhibit neural and non-neural uptake of catecholamines, respectively, and ascorbic acid (10 mM) to prevent the oxidation of catecholamines and was saturated with a mixture of 95% O_2 and 5% CO_2 at 37°C. After equilibration of the strips in normal PSS for 60 min, the responses to an agonist were recorded isometrically under an initial tension of 100 m*g*. The contraction response curves of norepinephrine were determined after sufficient repetitions for the curves to become constant. The pD_2 values for norepinephrine were expressed as negative logarithms of EC_{50}.

2.2. Measurement of cytosolic Ca^{2+} and tension in fura-2-loaded preparations

The responses to an agonist were recorded isometrically under an initial tension of 100 mg. High-K^+ solution was made by replacing NaCl with equimolar KCl. These solutions were saturated with a 95% O_2 and 5% CO_2 mixture at 37°C and pH 7.4. The strips were incubated with 5 μM fura-2/AM in normal PSS for 4 h at room temperature in the presence of 0.2% Cremophor EL, then rinsed with the same medium for 15 min; experiments were performed with a double-wavelength excitation fluorimeter (CAF-100; Japan Spectroscopic, Tokyo, Japan). Mechanical activity was monitored isometrically, and the ratio of 500 nm fluorescence emitted by 340 nm excitation (F_{340}) to that emitted by 380 nm excitation (F_{380}) was simultaneously calculated automatically from successive illumination periods (48 H_Z) and is referred to as $Ratio_{340/380}$ ($R_{340/380}$). In the muscle strips loaded with fura-2, the increase in cytosolic Ca^{2+} level resulted in an increase in F_{340}, a decrease in F_{380} and an increase in $R_{340/380}$. The $R_{340/380}$ was used to monitor the relative cytosolic Ca^{2+} level, $[Ca^{2+}]_i$ (Karaki et al., 1988; Sato et al., 1988).

2.3. Statistics

Numerical results are expressed as mean ± S.E., and statistical significance was calculated with Student's *t*-test. A P value less than 0.05 was considered to indicate a significant difference.

2.4. Drugs

The following drugs and chemicals were used: desmethylimipramine hydrochloride, (±)-normethanephrine hydrochloride, (±)-propranolol hydrochloride and yohimbine hydrochloride, (Sigma Chemicals, St. Louis, MO, USA), phorbol 12,13-dibutyrate (PDB), 4α-phorbol 12,13-dibutyrate (4α-PDB) (Biomol Research Laboratories, Gaithersburg, MD, USA), 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinecarboxylic acid methyl isopropyl ester (isradipine) (Research Biochemicals International, Natick, MA, USA), 1-[β-[3-(4-methoxyphenyl)propoxy]-4-metoxyphenethyl]-1*H*-imidazole (SKF-96365) hydrochloride (Calbiochemicals, San Diego, CA, USA), 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7), *N*-(2-cinnamylaminoethyl)-5-isoquinolinesulfonamide (H-88) (Seikagaku, Tokyo, Japan), norepinephrine hydrochloride, wortmannin, papaverine hydrochloride, 2-(1-(3-dimethylaminopropyl)-indol-3-yl)-3-(-indol-3-yl)-maleimide (GF 109203X) (Wako Pure Chemical Industries, Osaka, Japan), fura-2 pentaacetoxymethylester (fura-2/AM), ethyleneglycolbis (β-aminoethylether)*N*,*N*′-tetraacetic acid (EGTA), *N*-2-hydroxyethylpiperazine-*N*′-ethanesulphonic acid (HEPES), *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN) (Dojindo Laboratories, Kumamoto, Japan) and Cremophor EL (Nacalai Tesque, Kyoto, Japan). Other chemicals used were of analytical grade.

3. Results

3.1. Inhibitory effects of PDB on norepinephrine-induced contraction in rabbit iris dilator

Application of 10^{-4} M norepinephrine produced a contraction of strips which lasted over one h (Fig. 1A). The protein kinase C activating agent, PDB, relaxed the sustained contraction induced by norepinephrine in a concentration-dependent manner, but the inactive isomer of PDB, 4α-PDB, did not cause relaxation of the sustained contraction (Fig. 1B,E). The amplitude of this relaxation produced by PDB, 100% of which represents norepinephrine-induced sustained contraction, was 25.4 ± 2.46% ($n = 10$). The sustained contraction induced by norepinephrine was inhibited by pretreatment with 10^{-6} M PDB, and a transient contraction appeared (Fig. 1C). The magnitude of the sustained contraction was 31.4 ± 4.77% ($n = 4$). These residual contractions on treatment with PDB or a specific

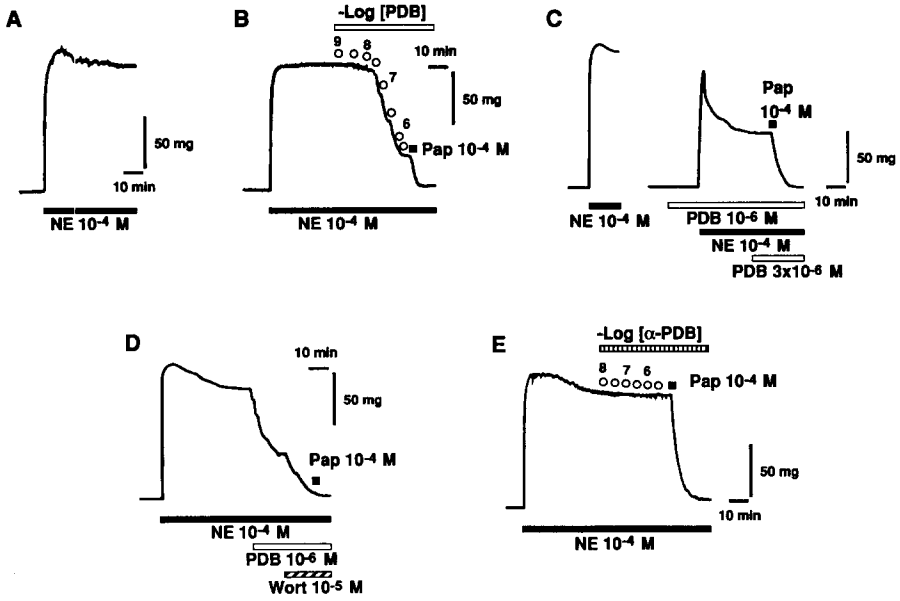

Fig. 1. Typical traces of contraction induced by norepinephrine (A) and the effects on it of phorbol 12,13-dibutyrate (PDB) (B,C), wortmannin (D) and 4α-PDB (E) in rabbit iris dilator. Norepinephrine (NE 10^{-4} M) was applied as indicated by the bar (A–E). PDB (B) and 4α-PDB (E) were cumulatively added to the preparation. Wortmannin (Wort 10^{-5} M) was applied as indicated by the hatched bar, and PDB (10^{-6} or 3×10^{-6} M) as indicated by an open bar. Papaverine (Pap 10^{-4} M) was added to obtain maximum relaxation (B–E).

myosin light chain kinase inhibitor, wortmannin, were relaxed by papaverine (10^{-4} M) to the resting tension (Fig. 1D).

3.2. Effect of PDB, isradipine and SKF-96365 on the concentration-response curves for norepinephrine

The pretreatment of iris dilator strips with each concentration of PDB decreased the maximum response in concentration-response curves for norepinephrine (Fig. 2A). The percentage of maximum contraction for norepinephrine with PDB (10^{-7} M) pretreatment was not significantly different from that obtained from the PDB (10^{-6} M)-pretreated strips, the maximum amplitude of this response being $44.3 \pm 0.08\%$ ($n = 5$). A potent L-type Ca^{2+} channel antagonist, isradipine (10^{-6} M), whose concentration was sufficient to block Ca^{2+} channels, did not influence the concentration-response curves of norepinephrine. A non-specific Ca^{2+} channel antagonist, SKF-96365 (10^{-4} M), shifted the curve for norepinephrine to the right and decreased the maximum response to this drug (Fig. 2B).

3.3. Effects of PDB on changes in cytosolic Ca^{2+} levels and contractions induced by norepinephrine and GF 109203X, H-7 or H-88 on PDB-induced changes in cytosolic Ca^{2+} levels and contractions induced by norepinephrine

As shown in Fig. 3A, norepinephrine (10^{-4} M) caused both a transient and a sustained increase in $[Ca^{2+}]_i$ ($R_{340/380}$) and increased muscle contraction. K^+-PSS at a concentration of 70 mM also caused a rapid increase in $[Ca^{2+}]_i$ ($R_{340/380}$) and the muscle contraction induced by norepinephrine reached a plateau; application of PDB (10^{-6} M) caused a decrease in $[Ca^{2+}]_i$ ($R_{340/380}$) as well as relaxation of muscle contraction induced by norepinephrine (Fig. 3B). With PDB pretreatment the rapid transient increases in $[Ca^{2+}]_i$ ($R_{340/380}$) and muscle tension were not affected but the sustained increases in $[Ca^{2+}]_i$ ($R_{340/380}$) and muscle tension were smaller than those obtained in non-pretreated strips (Fig. 3C–E). Potent protein kinase C inhibitors, GF 109203X (10^{-6} M), and H-7 (3×10^{-6} M) antagonized the PDB-produced decrease in $[Ca^{2+}]_i$ ($R_{340/380}$) and the relaxation of muscle contraction induced by norepinephrine (Figs. 4 and 5). Norepinephrine-induced muscle contraction was reduced by the treatment with H-7 (3×10^{-6} M) before and after application of norepinephrine (Fig. 5). However, as shown in Fig.

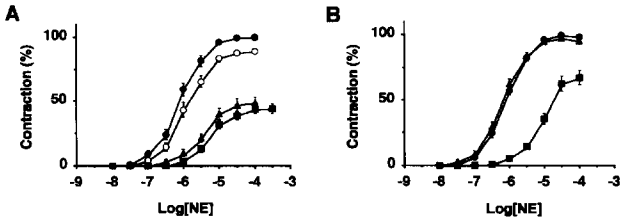

Fig. 2. Effects of phorbol 12,13-dibutyrate (PDB) (A) and Ca^{2+} channel antagonists (B) on norepinephrine (NE)-induced contractions in rabbit iris dilator. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by NE (10^{-4} M). Abscissa: log concentration (M) of NE. Vertical bars represent S.E. of 5 or 6 experiments. In A, ●, NE alone; ○, 10^{-8} M PDB; ▲, 10^{-7} M PDB; ■, 10^{-6} M PDB. In B, ●, NE alone; ▲, 3×10^{-6} M isradipine; ■, 10^{-4} M SKF 96365.

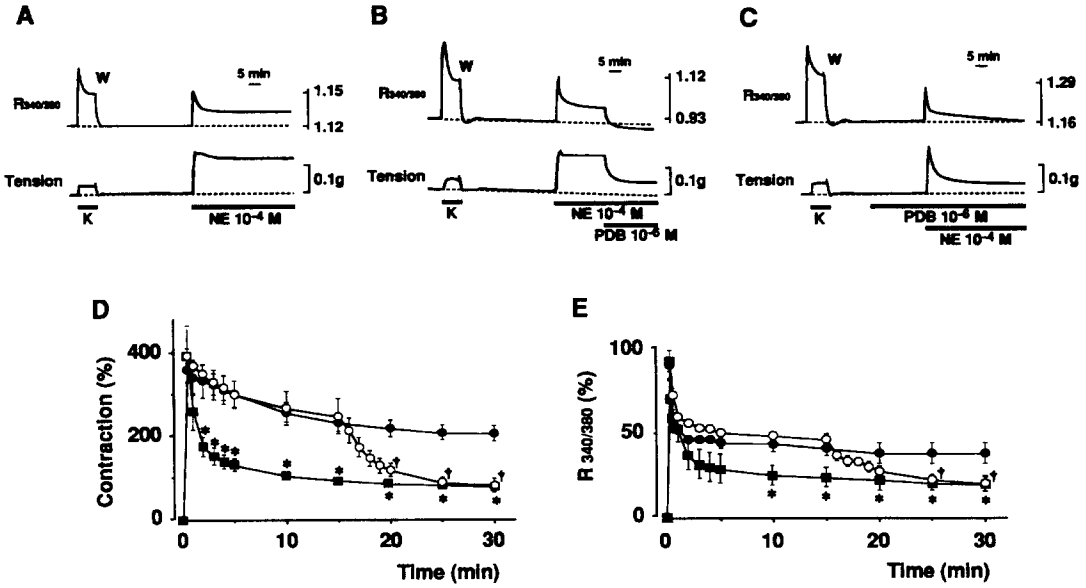

Fig. 3. Effects of phorbol 12,13-dibutyrate (PDB 10^{-6} M) on increases in $R_{340/380}$ ($[Ca^{2+}]_i$) and muscle tension induced by norepinephrine (NE 10^{-4} M) and measured simultaneously in fura-2-loaded strips. In A–C, the traces shown are typical of 4–6 experiments. Preparations were contracted with 70 mM KCl physiological saline solution (K^+-PSS) (K) as indicated by the line. In D, representative recordings of the time course of the changes in muscle tension induced by NE and PDB in NE-induced contraction are shown. E shows representative recordings of the time course of the changes in $[Ca^{2+}]_i$ induced by NE and PDB in the NE-induced increase in $[Ca^{2+}]_i$. Ordinate: contraction or $R_{340/380}$ 100% of which represents K^+-PSS-induced responses. Abscissa: time (min). ●, NE alone; ○, 10^{-6} M PDB; ■, NE with the pretreatment of 10^{-6} M PDB. Vertical bars represent S.E. from 4–6 experiments. *,† Significantly different from corresponding value ($P < 0.05$).

6, a potent protein kinase A inhibitor, H-88 (10^{-6} M) did not affect the PDB-induced decrease in $[Ca^{2+}]_i$ ($R_{340/380}$) or the relaxation of muscle contraction induced by norepinephrine.

3.4. $[Ca^{2+}]_i$ ($R_{340/380}$)-tension relationship

As shown in Fig. 7, there was a positive correlation between $[Ca^{2+}]_i$ ($R_{340/380}$) and tension for norepinephrine

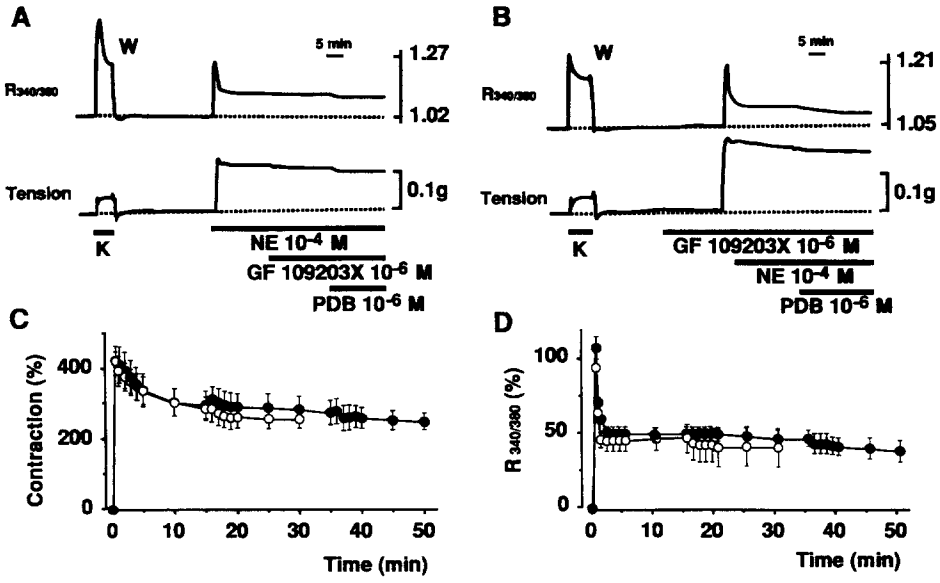

Fig. 4. Antagonistic effect of GF 109203X on phorbol 12,13-dibutyrate (PDB 10^{-6} M)-induced changes of $R_{340/380}$ ($[Ca^{2+}]_i$) and muscle tension measured simultaneously in fura-2-loaded strips. In A and B, the traces shown are typical of 6 experiments. Preparations were contracted by 70 mM KCl physiological saline solution (K^+-PSS) (K) as indicated by the line. C shows representative recordings of the time course of the effect of GF 109203X on the changes in the muscle tension induced by norepinephrine (NE) and of PDB on NE-induced contraction. D shows representative recordings of the time course of the effect of GF 109203X on the changes in $[Ca^{2+}]_i$ induced by NE or of PDB on NE-induced increase of $[Ca^{2+}]_i$. Ordinate: contraction and $R_{340/380}$ 100% of which represents K^+-PSS-induced responses. Abscissa: time (min). GF 109203X (10^{-6} M) was applied after (●) or before (○) application of NE (10^{-4} M). Vertical bars represent S.E. of 4–6 experiments.

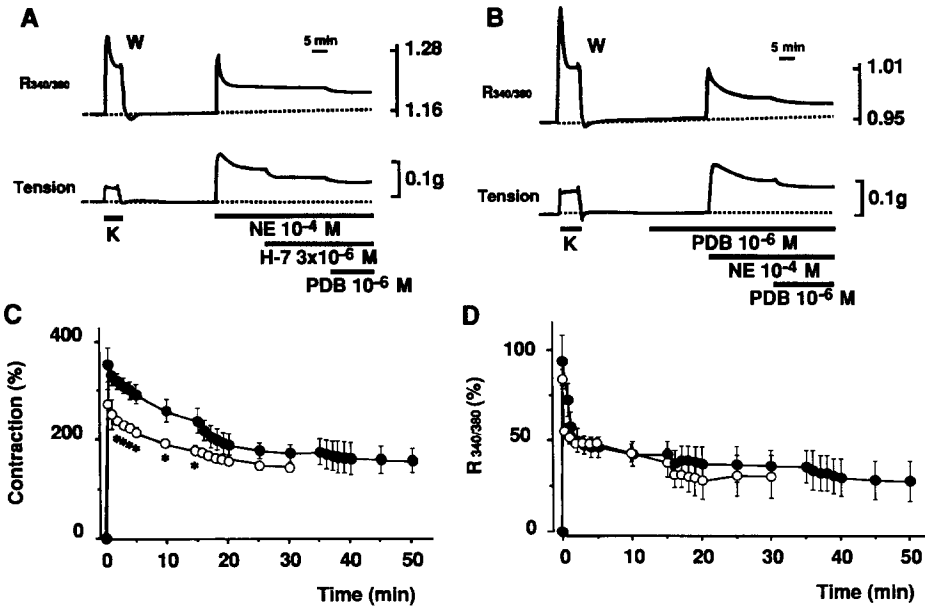

Fig. 5. Antagonistic effect of H-7 on phorbol 12,13-dibutyrate (PDB 10^{-6} M)-induced changes of $R_{340/380}$ ($[Ca^{2+}]_i$) and muscle tension measured simultaneously in fura-2-loaded strips. The traces shown in A and B are typical of 6 experiments. Preparations were contracted with 70 mM KCl physiological saline solution (K^+-PSS) (K) as indicated by the line. C shows representative recordings of the time course of the effect of H-7 on the changes in muscle tension induced by norepinephrine (NE) and PDB on NE-induced contraction, while D shows representative recordings of the time course of the effect of H-7 on the changes in $[Ca^{2+}]_i$ induced by NE or PDB on NE-induced increase of $[Ca^{2+}]_i$. Ordinate: contraction and $R_{340/380}$ 100% of which represents K^+-PSS-induced response. Abscissa: time (min). H-7 (3×10^{-6} M) was applied after (●) or before (○) application of NE (10^{-4} M). Vertical bars represent S.E. of 4–6 experiments. * Significantly different from corresponding value ($P < 0.05$).

and high K^+. The regression line for $[Ca^{2+}]_i$ ($R_{340/380}$) and tension was obtained using all the points for each stimulation. The slopes of the regression lines were 3.46 ± 0.59 ($n = 6$) for norepinephrine and 0.94 ± 0.027 ($n = 3$) for high K^+, respectively. The slope for norepinephrine was significantly steeper than that obtained with high K^+,

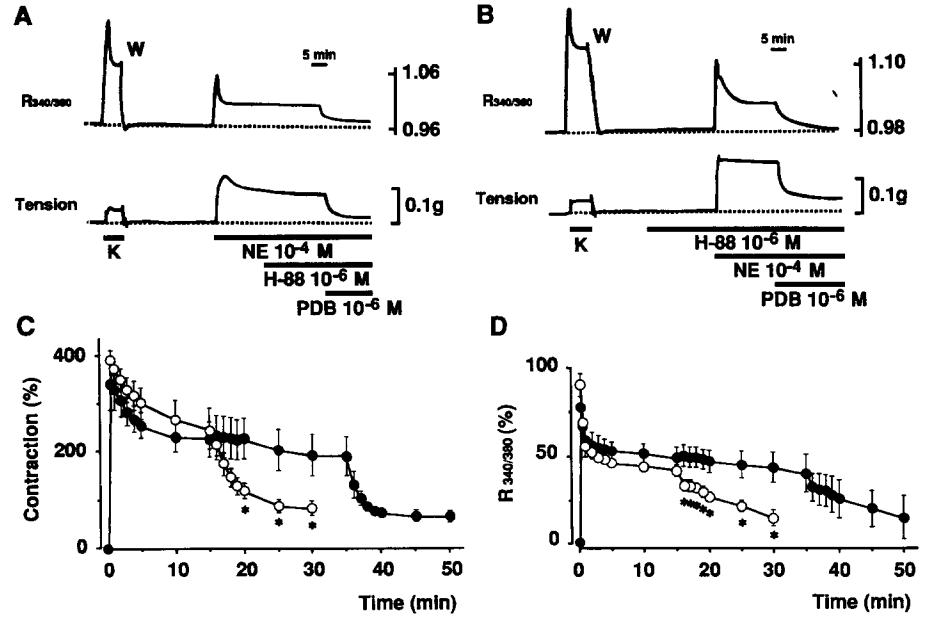

Fig. 6. Influence of H-88 on phorbol 12,13-dibutyrate (PDB 10^{-6} M)-induced changes of $R_{340/380}$ ($[Ca^{2+}]_i$) and muscle tension measured simultaneously in fura-2-loaded strips. The traces shown in A and B are typical of 6 experiments. Preparations were contracted with 70 mM KCl physiological saline solution (K^+-PSS) (K) as indicated by the line. C shows representative recordings of the time course of the effect of H-88 on the changes in muscle tension induced by norepinephrine (NE) and PDB on NE-induced contraction, while D shows representative recordings of the time course of the effect of H-88 on the changes in $[Ca^{2+}]_i$ induced by NE or PDB on NE-induced increase of $[Ca^{2+}]_i$. Ordinate: contraction and $R_{340/380}$ 100% of which represents K^+-PSS-induced responses. Abscissa: time (min). H-88 (10^{-6} M) was applied after (●) or before (○) application of NE (10^{-4} M). Vertical bars represent S.E. of 4–6 experiments. * Significantly different from corresponding value ($P < 0.05$).

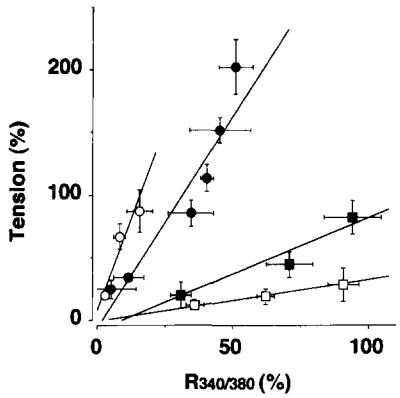

Fig. 7. Relationship between $R_{340/380}$ ($[Ca^{2+}]_i$) and tension development for norepinephrine and high K^+. ●, norepinephrine; ■, high K^+; ○, norepinephrine with PDB (10^{-6} M) pretreatment; □, high K^+ with PDB (10^{-6} M) pretreatment. Ordinate and abscissa, tension (%) and $R_{340/380}$ (%), 100% of which represents 70 mM K^+-induced responses. Each point represents the mean ± S.E. of 3–4 experiments.

indicating that Ca^{2+} utilization in the contractile response through α_1-adrenoceptors was higher than in K^+-induced responses. The slopes of regression lines obtained from PDB (10^{-6} M)-pretreated preparations were, respectively, 5.19 ± 1.74 ($n = 3$) for norepinephrine and 0.31 ± 0.020 ($n = 3$) for high K^+.

4. Discussion

Activation of α_1-adrenoceptors by norepinephrine has been reported to activate phospholipase C, leading to production of inositol phosphates and diacylglycerol (Berridge, 1984; Suzuki et al., 1990). Inositol 1,4,5-triphosphate (IP_3) is well recognized to mobilize intracellular Ca^{2+} from Ca^{2+} stores, whereas diacylglycerol activates some isoforms of protein kinase C. In rabbit iris dilator, norepinephrine caused rapid (transient) and long-lasting (sustained) increases in $[Ca^{2+}]_i$ and muscle contraction. In the present experiments, these responses (sustained increase in $[Ca^{2+}]_i$ and muscle contraction) were inhibited by the protein kinase C activator, PDB, and antagonized by potent protein kinase C inhibitors, GF 109203X and H-7 yet not by the protein kinase A inhibitor, H-88. In this smooth muscle, the contraction induced by norepinephrine does not depend functionally on the Ca^{2+} influx through Ca^{2+} channels. As shown in Fig. 2B, a potent voltage-dependent Ca^{2+} channel antagonist, isradipine, did not influence the concentration-response curves for norepinephrine, and a non-specific or receptor operated Ca^{2+} antagonist inhibited the concentration-response curve for norepinephrine at a high concentration. A specific inhibitor of Ca^{2+}-ATPase cyclopiazonic acid produces contraction of iris dilator muscle (Satoh et al., 1996), and reduces the increase of $[Ca^{2+}]_i$ induced by norepinephrine. Takayanagi et al. (1992) and Kokubu et al. (1993) found that the activation of α_{1B}-adrenoceptors induced by norepinephrine produced muscle contraction in rabbit iris dilator muscle. Suzuki et al. (1990) reported that activation of α_{1B}-adrenoceptors activated phospholipase C, and that this increased the intracellular Ca^{2+} concentration, resulting in the production of IP_3. These observations mirror our own findings in the present experiments. Kokubu et al. (1995) observed that protein kinase C activity was induced through α_1-adrenoceptors in the rabbit thoracic aorta and others also observed rapid and sustained protein kinase C activity, which regulates either phospholipase C, its receptor or a receptor-coupling mechanism (Kurata et al., 1993; Xuan et al., 1994). Orellana et al. (1987) proposed that phospholipase C is inhibited by protein kinase C-dependent phosphorylation. Our finding, the reduction of $[Ca^{2+}]_i$ produced by PDB, may result in these effects of protein kinase C. On the other hand, protein kinase C may maintain the activity of Ca^{2+} channels in the elevation of $[Ca^{2+}]_i$ or $^{45}Ca^{2+}$ influx (Xuan et al., 1994), and phosphorylation of the L-type Ca^{2+} channel by protein kinase A may increase Ca^{2+} gating by increasing the probability of channel opening (Tsien et al., 1986). These mechanisms are important in the sustained contraction of smooth muscle, such as aorta. Rabbit iris dilator muscle contains voltage-dependent Ca^{2+} channels, because high K^+-PSS induced an increase in $[Ca^{2+}]_i$ and contraction, and both increases were completely blocked by voltage-dependent Ca^{2+} channel antagonists, nicardipine (10^{-6} M) and isradipine (10^{-6} M). However, as shown in Fig. 2B and reported by Kokubu et al. (1993), L-type Ca^{2+} channel antagonists did not affect the norepinephrine-induced contraction, suggesting that voltage-dependent Ca^{2+} channels may not operate during agonist-induced contraction of rabbit iris dilator muscle, or that the contraction induced by norepinephrine does not depend functionally on the Ca^{2+} influx through Ca^{2+} channels in this tissue. For this reason, the contraction produced through norepinephrine-induced activation of voltage-dependent Ca^{2+} channel by PDB in the present studies might not have been observed.

In addition to these Ca^{2+}-related mechanisms, the activation of protein kinase C by the application of phorbol ester can induce strong and long-lasting contraction without changing $[Ca^{2+}]_i$ in vascular smooth muscle (Jiang and Morgan, 1987). Satoh et al. (1992a,b, 1994) and Kokubu et al. (1995) reported that, in rabbit thoracic aorta, α_{1A}- and α_{1B}-adrenoceptor subtypes were activated by norepinephrine, and that the Ca^{2+} sensitization produced by α_{1A} subtypes is mediated through G-protein and protein kinase C, which plays an important role in the contraction of smooth muscle of rabbit thoracic aorta. Using fura-2-loaded aortic smooth muscle, the slopes of the regression lines for $[Ca^{2+}]_i$ ($R_{340/380}$) and tension were steeper with norepinephrine and phenylephrine than the slope obtained from the high K^+-induced response (Satoh et al., 1995; Takayanagi and Onozuka, 1990). In the present experiments, as shown by relationship between $[Ca^{2+}]_i$ ($R_{340/380}$)

and tension development in Fig. 7, the slope of the regression line for norepinephrine in rabbit iris dilator was also steeper than the slope obtained from high K^+-induced responses, indicating that the Ca^{2+} sensitization during the sustained contraction of this muscle produced by norepinephrine was operating in the rabbit iris dilator. These findings were also supported by the observation that norepinephrine induced a much greater contraction than did high K^+ although norepinephrine induced a smaller increase in $[Ca^{2+}]_i$ than did high K^+ (Figs. 3–6). Based on the increase in the slope of the relationship between $[Ca^{2+}]_i$ and tension for norepinephrine after pretreatment with the protein kinase activator, PDB, the Ca^{2+} sensitivity for norepinephrine-induced contraction was increased. These findings suggest that agonists may increase the Ca^{2+} sensitivity of contractile elements by increasing the Ca^{2+} sensitivity of myosin light chain phosphorylation. Nishimura and Van Breemen (1989) provided evidence that receptor-mediated GTP-binding protein-coupled activation of protein kinase C increases the Ca^{2+} sensitivity of contractile elements. Karaki (1989) also mentioned that the contractility of vascular smooth muscle induced by receptor stimulants is regulated, not only by the increase in $[Ca^{2+}]_i$, but also by the increase in the Ca^{2+} sensitivity of the contractile elements. The suggestion that receptor-mediated GTP-binding protein-coupled activation of protein kinase C increases the Ca^{2+} sensitivity of contractile elements was also made by some investigators (Hirata et al., 1992; Hori et al., 1992; Kitazawa et al., 1991). And as shown in Figs. 1–3, PDB did not completely relax the muscle tension to its resting level (approximately 70%). One explanation of this finding may be that there is Ca^{2+} sensitization produced by protein kinase C in this muscle.

In this study, we have investigated the effect of protein kinase C on norepinephrine-induced mobilization of Ca^{2+} and contraction in rabbit iris dilator muscle. Although there are many substrates for protein kinase C, and several of the isoforms and cellular responses to activation of these kinases are complex, it appears that protein kinase C is important in the regulation of intracellular Ca^{2+} concentration mediated by inositol phosphate-dependent signaling events.

Acknowledgements

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