α -Adrenoceptor Agonists Produce Ca^{2+} Oscillations in Isolated Rat Aorta : Role of Protein Kinase C

Hiroshi SUENAGA and Katsuo KAMATA

*Department of Physiology and Morphology, Institute of Medicinal Chemistry,
Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan*

Abstract

We investigated the relationship between tension development and the cytosolic free Ca^{2+} level ($[\text{Ca}^{2+}]_i$) in responses to norepinephrine (NE) and selective α_2 -adrenoceptor agonist, UK14,304 of the endothelium-denuded rat aorta loaded with fura PE-3. NE (3×10^{-8} M) evoked a rapid increase in $[\text{Ca}^{2+}]_i$, followed by slight decreasing to a steady state level and produced a contraction. After the NE-induced increase in $[\text{Ca}^{2+}]_i$ had reached a maximum, the $[\text{Ca}^{2+}]_i$ showed persistent oscillations. The Ca^{2+} oscillations were superimposed on the sustained increase in $[\text{Ca}^{2+}]_i$. UK14,304 (3×10^{-6} M) also evoked an increase in $[\text{Ca}^{2+}]_i$ and produced a contraction. However, the UK14,304-induced effect on $[\text{Ca}^{2+}]_i$ was characterized by pronounced oscillations, and the amplitude of the sustained increase in $[\text{Ca}^{2+}]_i$ was less than that seen with NE. Protein kinase C inhibitor, Ro31-8220 (3×10^{-6} M) and verapamil (10^{-5} M) abolished both NE- and UK14,304-evoked Ca^{2+} oscillations. UK14,304-induced contractions were also strongly inhibited by Ro31-8220 and verapamil. However, NE-induced contractions were partly inhibited by these inhibitors. The sustained increases in $[\text{Ca}^{2+}]_i$ evoked NE and UK14,304 were not significantly inhibited by Ro31-8220 and verapamil. These results suggest that NE and UK14,304 produce Ca^{2+} oscillations during sustained contractions in rat aorta. The α_2 -adrenoceptor agonist, UK14,304-induced sustained contraction and Ca^{2+} oscillations may be due to PKC activation and opening of voltage-dependent L-type Ca^{2+} channels.

Key words: α -Adrenoceptor, UK14,304, norepinephrine, cytosolic free Ca^{2+} level, protein kinase C, rat aorta

Introduction

It is well known that α_2 -adrenoceptor activation in vascular smooth muscle produces a contraction that is closely related to the elevation of cytosolic free Ca^{2+} ($[\text{Ca}^{2+}]_i$). Activation of the α_1 -adrenoceptor is linked to the hydrolysis of polyphosphoinositide into inositol 1, 4, 5-trisphosphate (IP_3) and diacylglycerol (Villalobos-Molina *et al.* 1982; Rapoport, 1987; Tabernero *et al.* 1996). IP_3 can cause a Ca^{2+} release from sarcoplasmic reticulum and

Correspondence to : Katsuo Kamata, Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan
Phone : +81-3-5498-5856, Fax : +81-3-5498-5856
E-mail : kamata@hoshi.ac.jp

diacylglycerol activates protein kinase C (Berridge and Irvine, 1989; Nishizuka, 1992). In rat aorta, norepinephrine (NE)-induced sustained contraction has been reported to be strongly attenuated by removal of extracellular Ca^{2+} but is slightly inhibited by organic Ca^{2+} channel blockers (Beckeringh *et al.* 1984a; Chiu *et al.* 1987; Koch *et al.* 1990; Jenkin *et al.* 1991; Oriowo and Ruffolo, 1992). These reports suggest that NE-induced sustained contraction is due to Ca^{2+} influx through both L-type and non-L-type Ca^{2+} channels. As compared with NE response, α_2 -adrenoceptor agonists-induced contractions are known to be strongly dependent on Ca^{2+} influx through L-type Ca^{2+} channels in rat aorta (Beckeringh *et al.* 1984a; 1984b). Since α_2 -adrenoceptor agonists-induced contractions are involved in islet-activating protein (IAP)-sensitive GTP-binding protein (Nichols *et al.* 1989; Suenaga *et al.* 1996), α_2 -adrenoceptor agonists may not directly open the Ca^{2+} channel. However, precise mechanism underlying α_2 -adrenoceptor agonists-induced contractile responses has not been defined.

It is well documented that protein kinase C (PKC) plays an important role in the contractile response of vascular smooth muscle (Nishizuka, 1984; Rasmussen *et al.* 1987). Phorbol esters (exogenous PKC activators) are able to produce a sustained contraction of vascular smooth muscle (Danthuluri and Deth, 1984; Rasmussen *et al.* 1984; 1987; Itoh and Lederis, 1987). We have previously reported that phorbol 12-myristate 13-acetate (PMA) produces biphasic contractions of the rat aorta by activation of Ca^{2+} -independent and Ca^{2+} -dependent contractile mechanisms (Suenaga *et al.* 1993), and that the Ca^{2+} -dependent contractile response is regulated by IAP-sensitive GTP-binding protein (Suenaga *et al.* 1996). Although PKC activation is able to produce a sustained contraction, there is little evidence concerning the relationship, if any, between this sustained contraction and the receptor stimulation-induced type of sustained contraction. It is reported that phenylephrine-induced contraction is slightly inhibited by a selective PKC inhibitor, calphostin C (Shimamoto *et al.* 1993). Moreover, PKC down-regulation by long-term exposure of vascular smooth muscle to phorbol ester has been reported to be no effect on the maximal NE-induced contraction (Hori *et al.* 1993). These reports suggest that the involvement of PKC activation in the phenylephrine- and NE-induced contractions is small. In contrast, it is reported that selective α_2 -adrenoceptor agonist, UK14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline)-induced responses are dependent on PKC activation in vascular smooth muscles (Aburto *et al.* 1995; Shimamoto *et al.* 1995).

It has been reported that spontaneous Ca^{2+} oscillations (repetitive transient increases in $[\text{Ca}^{2+}]_i$) or vasoactive hormones-induced Ca^{2+} oscillations were observed in cultured vascular smooth muscle cells (Byron and Taylor, 1993; Wu *et al.* 1995; Byron, 1996; Hamada *et al.* 1997). In isolated rat aorta, phenylephrine has also been reported to evoke a repetitive spike-like changes in $[\text{Ca}^{2+}]_i$ (Takayama *et al.* 1996). However, the relationship between the Ca^{2+} response with oscillation and contraction evoked by α -adrenoceptor activation is not fully explained.

In the present study, we investigated the role of PKC in the α -adrenoceptor-stimulated contractile response in rat aorta. We also measured the changes in intracellular free Ca^{2+} that occurred with the contractions evoked by α -adrenoceptor agonists.

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 strips

Male Wistar rats, 8–10 weeks old, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), then killed by decapitation. The thoracic aorta was rapidly dissected out and placed into modified Krebs-Henseleit solution (KHS, composition in mM: NaCl 118; KCl 4.7; CaCl_2 1.8; NaHCO_3 25.0; MgSO_4 1.2; NaH_2PO_4 1.2; dextrose 11.0). It was then cleaned of loosely adhering fat and connective tissue and cut into helical strips 2 mm in width and 10 mm in length. The endothelium was removed by rubbing the intimal surface with a cotton swab, successful removal being functionally confirmed by the absence of a relaxation to 10 μM acetylcholine.

Measurement of intracellular free Ca^{2+} and tension

Tension and cytosolic free Ca^{2+} ($[\text{Ca}^{2+}]_i$) were measured by the method of Sato *et al.* (1988) with the minor modifications described previously (Suenaga and Kamata, 1998). The aortic strips were exposed to 10^{-5} M fura PE3-acetoxymethyl ester (fura PE3-AM) in the presence of 0.04% cremophor EL in the dark for 5 h at 26 °C. The tissue was then rinsed with normal KHS and placed in the organ bath of a fluorometer (Japan Spectroscopic, CAF 110, Tokyo, Japan) containing KHS at 37 °C. The tissue was held in a horizontal configuration under a resting tension of 0.5 g (which is the optimal tension for inducing a maximal contraction) and equilibrated for 30 min. One end of the tissue was connected to a force transducer for the measurement of isometric tension (Nihon Kohden, TB-611, Tokyo, Japan) and the other end was fixed. The intimal surface of the fura-PE3-loaded tissue was alternately (frequency, 128 Hz) subjected to excitation wavelengths of 340 and 380 nm. The fluorescence emissions at a wavelength of 500 nm for each excitation light were separated by means of a dichroic mirror and measured with the aid of a photomultiplier. The ratio (F_{340}/F_{380}) of the emitted fluorescence signals was used to provide an index of $[\text{Ca}^{2+}]_i$. The contractile responses and the emitted fluorescence signals were displayed on a pen recorder (Yokogawa, Model 3021, Tokyo, Japan).

After equilibration, each aortic strip was first exposed to 80 mM K^+ (made by iso-osmotic substitution for NaCl). The fluorescence ratio (F_{340}/F_{380}) in the resting muscle and that in muscle depolarized by 80 mM K^+ were taken as 0 and 100%, respectively. After washing, the aortic strips were treated with Ro31-8220 ($\{1-[3-(\text{amidinothio})\text{propyl}-1\text{h-indoyl}-3\text{-yl}]-3-(1\text{-methyl-1h-indoyl}-3\text{-yl})\text{-maleimide-methane sulfate}\}$), H-7 ($1-\{5\text{-isoquinolinesulfonyl}\}-2\text{-methylpiperazine dihydrochloride}$) or verapamil for 15 min and then high K^+ , NE or UK14,304 was applied.

Effects of PKC inhibitor, Ca²⁺ channel blocker and α -adrenoceptor antagonists on vascular contraction

Endothelium-denuded aortic strips (without fura PE3-loading) were first contracted by 80 mM K⁺ and these responses were taken as 100%. After washing and equilibrating for 1 h, the aortic strips were treated with Ro31-8220, verapamil, prazosin or yohimbine and then high K⁺, NE or UK14,304 was cumulatively applied.

Drugs

Cremophor EL, H-7, NE, prazosin, verapamil and yohimbine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetylcholine chloride was purchased from Daiichi Pharmaceuticals Co. (Tokyo, Japan). Fura PE3-AM and Ro31-8220 were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). UK14,304 was purchased from Funakoshi Co. (Tokyo, Japan). Phentolamine methanesulfonate was purchased from Ciba-Geigy (Hyogo, Japan). Fura PE3-AM was dissolved in dimethylsulfoxide (DMSO) and mixed with cremophor EL. The final concentrations of DMSO and cremophor EL were 1% and 0.04%, respectively. At these concentrations, DMSO and cremophor EL had no detectable effect on [Ca²⁺]_i or tension in the aortic strips. Prazosin, Ro31-8220 and UK14,304 were dissolved in DMSO and diluted in distilled water. All the other drugs were dissolved in distilled water.

Statistics

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. Antagonist potency was expressed as a pK_B from the equation $\text{pK}_B = B + \log(\text{DR} - 1)$, where B is the antagonist concentration ($-\log M$), and DR is the agonist dose ratio.

Results

Effect of α -adrenoceptor agonists on cytosolic Ca²⁺ and tension

Typical changes evoked by NE and UK14,304 in muscle tension development and intracellular free Ca²⁺ ([Ca²⁺]_i) in fura PE-3 loaded aortic strips are illustrated in Fig. 1. NE (3×10^{-8} M) evoked a rapid increase in [Ca²⁺]_i followed by slight decreasing to a steady state level. After the NE-induced increase in [Ca²⁺]_i had reached a maximum, the intracellular free Ca²⁺ showed persistent oscillations. The Ca²⁺ oscillations were superimposed on the sustained increase in [Ca²⁺]_i. The frequency of the oscillations was 0.39 ± 0.04 Hz ($n=7$). UK14,304 (3×10^{-6} M) also evoked an increase in [Ca²⁺]_i and produced a contraction (Fig. 1). The contractile response to 3×10^{-6} M UK14,304 was equal in amplitude to that evoked by 3×10^{-8} M NE. However, the UK14,304-induced effect on intracellular free Ca²⁺ was characterized by pronounced oscillations, and the amplitude of Ca²⁺ oscillations was significantly larger than that seen with NE. In contrast, the amplitude of the sustained increase in [Ca²⁺]_i evoked by UK14,304 was less than that seen with NE. The frequency of the oscillations evoked by 3×10^{-6} M UK14,304 was 0.38 ± 0.02 Hz ($n=6$). The increases in [Ca²⁺]_i (oscillations and sustained increase in [Ca²⁺]_i) and contractile responses induced by both 3×10^{-8} M NE and $3 \times$

Fig. 1. Typical traces showing changes in fluorescence ratio (F_{340}/F_{380}) and force development induced by 3×10^{-8} M NE, 3×10^{-6} M UK14,304 or 30 mM K^+ in the rat aorta. The resting level is taken as 0% and the 80 mM K^+ -stimulated level as 100%.

10^{-6} M UK14,304 were abolished by 10^{-6} M phentolamine. (data not shown). The application of 30 mM K^+ also evoked an increase in $[Ca^{2+}]_i$ and produced a contraction (Fig. 1). The contractile response to 30 mM K^+ was equal in amplitude to that evoked by 3×10^{-8} M NE. However, Ca^{2+} oscillations were not evoked. Application of several concentrations of high K^+ (10, 15, 20 mM) also did not evoke Ca^{2+} oscillations (data not shown). Figure 2

Fig. 2. Changes in $[Ca^{2+}]_i$ level and muscle tension in response to 3×10^{-8} M NE, 3×10^{-6} M UK14,304 or 30 mM K^+ in the rat aorta. The resting level is taken as 0% and the 80 mM K^+ -stimulated level as 100%. The increment in the $[Ca^{2+}]_i$ level (measured at maximal contraction) divided into the sustained level (sustained Ca^{2+}) and the size of the oscillation (Ca^{2+} oscillation). Values represent the mean $\pm$ S.E. of 5-7 muscle preparations, each isolated from a different animal. ** $p < 0.01$ significantly different from the values of NE.

Fig. 3. Effects of various concentration of UK14,304 on oscillations in $[Ca^{2+}]_i$ (Ca^{2+} oscillation), sustained increase in $[Ca^{2+}]_i$ (sustained Ca^{2+}), contraction in the rat aorta. The resting level as 0% and the 80 mM K^+ -stimulated level as 100%. Right columns show the frequency of the Ca^{2+} oscillations. Values represent the mean $\pm$ S.E. from 5-7 muscle preparations, each isolated from a different animal.

shows a summary of the changes in $[Ca^{2+}]_i$ (oscillations and sustained level) and contractions evoked by 3×10^{-8} M NE, 3×10^{-6} M UK14,304 and 30 mM K^+ .

Figure 3 shows the changes in $[Ca^{2+}]_i$ and contraction induced by various concentrations of UK14,304. UK14,304 at a concentration of 10^{-7} M produced only a sustained increases in $[Ca^{2+}]_i$, but it's higher concentrations evoked Ca^{2+} oscillations. Both the amplitude of Ca^{2+} oscillations and contractile response were dependent on the concentration of UK14,304. Since the amplitude of the sustained increases in $[Ca^{2+}]_i$ evoked by UK14,304 at a concentration of 10^{-7} M to 10^{-5} M were very small, the responses were not found to vary with the concentration of UK14,304. The frequency of the Ca^{2+} oscillations was also dependent on the concentration of UK14,304 (Fig. 3).

Effects of PKC inhibitors and α -adrenoceptor antagonists on α -adrenoceptor agonists-induced responses

Figure 4 shows the effect of Ro31-8220 on the dose-response curves for NE and UK14,304 in the rat aorta (without fura PE3-loading). Ro31-8220, at a concentration of 3×10^{-7} M, did not affect the NE-induced contraction. Higher concentrations of Ro31-8220 were dose dependently inhibited NE-induced contractions. However, the inhibitory effect of Ro31-8220 was only moderate. Ro31-8220 (3×10^{-6} M) reduced the contractile response to 10^{-5} M NE by 15.4% (from $101.4 \pm 1.0\%$ to $85.8 \pm 1.5\%$ of the 80 mM K^+ -induced response). In contrast, the UK14,304-induced contraction was markedly inhibited by Ro31-8220, these effects being greater than that on the NE-induced contraction. Ro31-8220 (3×10^{-6} M) reduced the contractile response to 3×10^{-5} M UK14,304 by 93.6% (from $91.8 \pm 2.5\%$ to $5.9 \pm 1.6\%$ of the 80 mM K^+ -induced response). Dose-response curve for high K^+ was not inhibited by 3×10^{-6} M Ro31-8220 (data not shown). The dose-response curve for UK14,304 was shifted to the right by α -adrenoceptor antagonists prazosin (10^{-9} M) and yohimbine (10^{-6} M). The prazosin and yohimbine pK_B values were 9.64 ± 0.14 ($n=5$) and 6.62 ± 0.04 ($n=5$), respectively.

In fura PE-3 loaded aorta, both amplitude of the Ca^{2+} oscillations and the contractile response induced by 3×10^{-6} M UK14,304 were inhibited by Ro31-8220, in a dose dependent

Fig. 4. Dose-dependent response of aortic strips to NE and UK14,304 in the absence (control) or presence of various concentrations of Ro31-8220 (Ro). Each point is the mean from 4-6 muscle preparations, each isolated from a different animal; vertical lines indicating $\pm$ S.E. are only shown when they exceed the dimensions of the symbol used. Values are expressed as a percentage of the tension developed as a result of pretreatment with 80 mM K^+ in each strip. * $p < 0.05$, ** $p < 0.01$ vs. control in the absence of Ro31-8220.

Fig. 5. Effects of various concentration of Ro31-8220 (Ro) and 10^{-5} M H-7 on oscillations in $[Ca^{2+}]_i$ (Ca^{2+} oscillation), sustained increase in $[Ca^{2+}]_i$ (sustained Ca^{2+}) and contraction evoked by 3×10^{-6} M UK14,304 in the rat aorta. The resting level as 0% and the 80 mM K^+ -stimulated level as 100%. Values represent the mean $\pm$ S.E. from 4-7 muscle preparations, each isolated from a different animal. * $p < 0.05$, ** $p < 0.01$ vs. control in the absence of Ro31-8220 and H-7.

manner (Fig. 5). However, the sustained increase in $[Ca^{2+}]_i$ evoked by UK14,304 was not significantly inhibited by Ro31-8220. The Ca^{2+} oscillations and the contractile response induced by 3×10^{-6} M UK14,304 were also inhibited by 10^{-5} M H-7 (Fig. 5). However, the sustained increase in $[Ca^{2+}]_i$ was not significantly inhibited by 10^{-5} M H-7.

Effect of Ca^{2+} channel blocker on α -adrenoceptor agonists-induced contractions

Figure 6 shows the effects of verapamil on the dose-response curves for NE and UK14,304 in the rat aorta (without fura PE3-loading). NE-induced contraction was slightly but significantly inhibited by 10^{-5} M verapamil. In contrast, the UK14,304-induced contraction was markedly inhibited by verapamil, this effect being greater than that on the NE-induced contraction.

Fig. 6. Dose-dependent response of aortic strips to NE and UK14,304 in the absence (control) or presence of 10^{-5} M verapamil. Each point is the mean from 4–5 muscle preparations, each isolated from a different animal; vertical lines indicating $\pm$ S.E. are only shown when they exceed the dimensions of the symbol used. Values are expressed as a percentage of the tension developed as a result of pretreatment with 80 mM K^+ in each strip. * $p < 0.05$, ** $p < 0.01$ vs. control in the absence of verapamil.

Effect of PKC inhibitor and Ca^{2+} channel blocker on changes in cytosolic Ca^{2+} and tension

Figure 7 shows the effects of Ro31-8220 and verapamil on the increases in $[Ca^{2+}]_i$ and contractions induced by 3×10^{-8} M NE, 3×10^{-6} M UK14,304 and 30 mM K^+ . Ro31-8220 (3×10^{-6} M) abolished Ca^{2+} oscillations and inhibited contractions induced by NE and UK14,304. However, the inhibitory effect of Ro31-8220 on UK14,304-induced contraction was greater than that on NE-induced contraction. NE- and UK14,304-induced sustained increases in $[Ca^{2+}]_i$ were not significantly inhibited by Ro31-8220. Ro31-8220 (3×10^{-6} M) did not affect the sustained increase in $[Ca^{2+}]_i$ and the contraction induced by 30 mM K^+ (Ca^{2+} oscillations were not evoked by 30 mM K^+).

NE- and UK14,304-induced Ca^{2+} oscillations were also abolished by 10^{-5} M verapamil. Verapamil significantly inhibited contractions induced by NE and UK14,304. However, inhibitory effect of verapamil on UK14,304-induced contraction was greater than that on the NE-induced contraction. NE- and UK14,304-induced sustained increase in $[Ca^{2+}]_i$ were not significantly inhibited by verapamil. Verapamil strongly inhibited 30 mM K^+ induced responses.

Discussion

In the present study, NE evoked oscillations in $[Ca^{2+}]_i$ during the sustained contraction in fura PE3 loaded rat aorta. The Ca^{2+} oscillations were superimposed on the sustained increase in $[Ca^{2+}]_i$. Verapamil abolished the Ca^{2+} oscillations and inhibited the contractile response. However, the sustained increase in $[Ca^{2+}]_i$ was not significantly inhibited by verapamil. These results suggest that NE may activate both L-type and non-L-type Ca^{2+} channels and that the Ca^{2+} oscillations may be dependent on Ca^{2+} influx via the voltage-dependent L-type Ca^{2+} channels in rat aorta. Similar results are previously reported that endothelin-1 induces Ca^{2+} oscillations which are inhibited by nifedipine in vascular smooth muscle cells (Simpson

Fig. 7. Effects of Ro31-8220 (3×10^{-6} M) and verapamil (10^{-5} M) on oscillations in $[Ca^{2+}]_i$ (Ca^{2+} oscillation), sustained increase in $[Ca^{2+}]_i$ (sustained Ca^{2+}) and contraction evoked by 3×10^{-8} M NE, 3×10^{-6} M UK14,304 or 30 mM K^+ in the rat aorta. The resting level as 0% and the 80 mM K^+ -stimulated level as 100%. Values represent the mean $\pm$ S.E. from 4-7 muscle preparations, each isolated from a different animal. ** $p < 0.01$ vs. control in the absence of Ro31-8220 and verapamil.

and Ashley, 1989). In the present study, NE-evoked Ca^{2+} oscillations, but not the sustained increase in $[Ca^{2+}]_i$ were also abolished by PKC inhibitor, Ro31-8220, suggesting that the generation of Ca^{2+} oscillations may be also involved in PKC activation in rat aorta.

Although NE-evoked Ca^{2+} oscillations were abolished by both Ro31-8220 and verapamil, NE-induced contractions were partly inhibited by both inhibitors in the present study. Moreover, the maximal contractions for cumulative addition of NE were only a slightly inhibited by both Ro31-8220 and verapamil. These results suggest that NE may also be able to produce PKC-independent contraction and that NE-evoked sustained increase in $[Ca^{2+}]_i$ may be at least partly involved in PKC-independent contraction in rat aorta. This speculation may be supported by the previous reports that (i) NE-induced contraction is strongly inhibited by removal of extracellular Ca^{2+} but it slightly inhibited by L-type Ca^{2+} channel blockers in rat aorta (Koch *et al.* 1990; Jenkin *et al.* 1991), suggesting that a part of NE-induced sustained contraction is due to Ca^{2+} influx through non-L-type Ca^{2+} channels and that (ii) PKC down-

regulation is not affect the NE-induced maximal contraction (Hori *et al.* 1993), suggesting that NE is able to produce PKC-independent contraction. In recent reports suggest that Rho-associated kinase is involved in the vascular contractions induced by some α -adrenoceptor agonists (Kokubu *et al.* 1995; Uehata *et al.* 1997).

In the present study, we found that the selective α_2 -adrenoceptor agonist, UK14,304 evoked pronounced oscillations in $[Ca^{2+}]_i$ during the sustained contraction and that the amplitude of the sustained increase in $[Ca^{2+}]_i$ was less than that seen with NE in rat aorta. Ro31-8220 and H-7 (another PKC inhibitor) abolished the Ca^{2+} oscillations and strongly inhibited the contraction. In contrast, the small sustained increase in $[Ca^{2+}]_i$ was resistant to these inhibitors. The dose-response curves for UK14,304, but not for NE was also strongly inhibited by Ro31-8220. These results suggest that UK14,304-induced contractile response may be principally due to PKC activation and that UK14,304 barely produce PKC-independent contraction in rat aorta. It is consider that PKC is one of major mechanisms producing a sustained contraction in vascular smooth muscles. However, recent studies suggest that PKC activation is minor role producing the sustained contraction evoked by α -adrenoceptor agonist in rat aorta (Hori *et al.* 1993; Shimamoto *et al.* 1993). The present study suggests that PKC-dependent oscillations in $[Ca^{2+}]_i$ were evoked by α -adrenoceptor agonists, NE and UK14,304 and that UK14,304-induced sustained contraction is strongly dependent on PKC activation in rat aorta. Since NE but not UK14,304 may be able to stimulate PKC-independent contractile mechanisms, NE-induced response is resistant to the PKC inhibitor. Because Ro31-8220 abolished UK14,304-evoked Ca^{2+} oscillations but it could not completely inhibit the contractile response, UK14,304-induced contraction is not entirely due to the increase in $[Ca^{2+}]_i$ with oscillations.

In the present study, verapamil also abolished UK14,304-evoked Ca^{2+} oscillations and strongly inhibited the contractile response. However, UK14,304-evoked small sustained increase in $[Ca^{2+}]_i$ was not significantly inhibited by verapamil. These results suggest that UK14,304-induced contractile response may be strongly dependent on Ca^{2+} influx via the voltage-dependent L-type Ca^{2+} channels and that UK14,304 barely activate non-L-type Ca^{2+} channels. It is well known that α_2 -adrenoceptor agonists-induced contractions are strongly dependent on Ca^{2+} influx through voltage-dependent Ca^{2+} channels in rat aorta (Beckeringh *et al.* 1984a; 1984b). Although an α_2 -adrenoceptor is expressed in rat aorta (Ping and Faber, 1993), it is generally accepted that some α_2 -adrenoceptor agonists-induced contractions in rat aorta are due to the partial agonistic properties of these compounds at α_1 -adrenoceptors (Digges and Summers, 1983; Beckeringh *et al.* 1984b; Macia *et al.* 1984; Wong, 1992; Moina *et al.* 1994). In the present study, the potency of prazosin (pK_B of 9.64) and yohimbine (pK_B of 6.62) suggest that contractions to UK14,304 in rat aorta involve mainly α_1 -adrenoceptors. It has been reported that partial α_1 -adrenoceptor agonists relay on the influx of extracellular Ca^{2+} , whereas full agonists (NE and phenylephrine) promote both Ca^{2+} influx and the release of intracellular Ca^{2+} (Chiu *et al.* 1987). The present results suggest that an α_2 -adrenoceptor agonist, UK14,304 evokes Ca^{2+} oscillations and produces a sustained contraction that are strongly dependent on both PKC activation and Ca^{2+} influx through voltage-dependent Ca^{2+} channels in rat aorta. However, the relationship between the generation of the Ca^{2+} oscillations and PKC activation is still unclear. Berrie and Cobbold (1995) have been reported that

PKC plays both negative and positive feedback roles in the hepatocyte $[Ca^{2+}]_i$ oscillator. The present results do not suggest the site of action of PKC on α -adrenoceptor agonists-induced Ca^{2+} oscillations. In vascular smooth muscle, PKC activation by phorbol ester has been reported to increase a dihydropyridine-sensitive calcium conductance (Fish *et al.* 1988). We previously reported that phorbol ester-induced contraction was partially inhibited by nicardipine in rat aorta (Suenaga *et al.* 1993). These reports suggest that activation of PKC promotes opening of voltage-dependent L-type Ca^{2+} channels. It has also been reported that spontaneous Ca^{2+} spikes are observed in cultured vascular smooth muscle and that high K^+ and Bay K 8644 increase the frequency of the Ca^{2+} spiking (Byron and Taylor, 1993). This report suggests that opening of voltage-dependent L-type Ca^{2+} channels is an essential feature of Ca^{2+} spiking generation. The present results also suggest that Ca^{2+} entry through voltage-dependent L-type Ca^{2+} channels is required for Ca^{2+} oscillations evoked by UK14,304. However, in the present study, spontaneous Ca^{2+} oscillations are not observed and application of high K^+ did not evoke Ca^{2+} oscillations, suggesting that opening of voltage-dependent L-type Ca^{2+} channels alone could not produce Ca^{2+} oscillations in isolated rat aorta. We consider that the mechanisms involved in both PKC activation and opening of voltage-dependent L-type Ca^{2+} channels are related to the generation of Ca^{2+} oscillations.

It is considered that Ca^{2+} oscillations are elicited by at least two different mechanisms, one that Ca^{2+} oscillations are mediated via the regenerative opening of voltage-dependent Ca^{2+} channels, which is accompanied with the periodic activation of the Ca^{2+} -activated K^+ channels (membrane oscillator), another that the Ca^{2+} oscillations are involved in the periodic Ca^{2+} release from the intracellular stores (cytosolic oscillator) (Byron and Taylor, 1993; Wu *et al.* 1995). We did not investigate the mechanism underlying the Ca^{2+} oscillations, but we consider that UK14,304-evoked Ca^{2+} oscillations may not be due to cytosolic oscillator. Because IP_3 -stimulated Ca^{2+} release from the intracellular stores is the key messenger for cytosolic oscillator (Hamada *et al.* 1997), but partial α_1 -adrenoceptor agonist does not promote the release of intracellular Ca^{2+} and does not stimulate the phosphoinositide hydrolysis in rat aorta (Chiu *et al.* 1987; Tabernero *et al.* 1996).

Although the results of the present study on $[Ca^{2+}]_i$ is due to average of the change in $[Ca^{2+}]_i$ in some smooth muscle cells, the Ca^{2+} oscillations were detected. This result suggests that the Ca^{2+} oscillations may be synchronized with cell population. It has been reported that synchronization of spontaneous Ca^{2+} spiking occur by cell to cell contact (gap junctions) in A7r5 cells (Byron and Taylor, 1993).

In conclusion, α -adrenoceptor agonists, UK14,304 and NE produce Ca^{2+} oscillations during sustained contractions in rat aorta. The α_2 -adrenoceptor agonist, UK14,304-induced sustained contraction and Ca^{2+} oscillations may be due to PKC activation and opening of voltage-dependent L-type Ca^{2+} channels.

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