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Effects of Phenylephrine on the Contractile Tension and Cytosolic Ca^{2+} Level in Rat Anococcygeus Muscle

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Abstract

A contractile propertiy of phenylephrine (PE), α_1 agonist, on rat anococcygeus muscle was compared with that on rat aorta by simultaneously measuring changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) level and muscle tension. (1) PE (0.1～30 μM) and high K^+ induced a sustained increases in $[Ca^{2+}]_i$ level and muscle tension of both the muscles. (2) An application of verapamil (10 μM) and EGTA (4 mM) decreased the PE- or high K^+-increased tension and $[Ca^{2+}]_i$ level in both the muscle, respectively. (3) A cumulative application of PE or high K^+ to anococcygeus muscle and aorta exhibited a positive relationship between $[Ca^{2+}]_i$ and developed tension. The developed tension by PE was greater than that by high K^+ at the same level of $[Ca^{2+}]_i$ only in the aorta. A difference of regression slopes in the relationship between $[Ca^{2+}]_i$ level and muscle tension under PE- and high K^+-treatments in aorta was significant, but that in anococcygeus muscle was not. (4) An application of PE to anococcygeus muscle in Ca^{2+} free medium elicited a small transient contractile tension and increase in $[Ca^{2+}]_i$ level, but that to aorta showed a large and transient increase in both the parameters. (5) Phorbol ester, DPB (1 μM), did not affect muscle tension or $[Ca^{2+}]_i$ level in anococcygeus muscle, but DPB induced greater increases in aorta. (6) An application of PE (10 μM) with GTP produced a left shift in the pCa-tension curve in the β-escin-permeabilized fiber of the anococcygeus muscle.

In summary, it is suggested that the sustained contraction induced by PE in anococcygeus muscle is involved with the increases in $[Ca^{2+}]_i$ which is due to Ca^{2+} influx mediated by α_1 receptor, but scarecely to Ca^{2+} release from the intracellular storage, and that an increase in Ca^{2+} sensitivity to PE is found only in the permeabilized anococcygeus muscle. The Ca^{2+}-independent contractile mechanism in PE response as seen in aorta is probably to be absent in anococcygeus muscle. Moreover, it seems that the effect of the drug acting protein kinase C on anococcygeus muscle is extremely lesser than that on aorta.

Key words: Phenylephrine, Anococcygeus muscle, Cytosolic Ca^{2+}, Ca^{2+} release, Protein kinase C

Introduction

It has been found that a muscle contraction is proceeded by elevating a cytosolic Ca^{2+} ($[Ca^{2+}]_i$) level which were measured using a fluorescent indicator, fura 2 in various kinds of smooth muscles. In rat vascular smooth muscle, an application of high K^+ or receptor agonist

induced sustained increments in muscle tension and $[Ca^{2+}]_i$ (Ozaki *et al.*, 1987; Sato *et al.*, 1988; Thorin-Threscases *et al.*, 1990), but receptor agonists induced a greater contraction than high K^+ for a given increase in $[Ca^{2+}]_i$ and it was indicated that the dissociation of the increase in $[Ca^{2+}]_i$ and muscle tension (Karaki *et al.*, 1988; Sato *et al.*, 1988; Takayanagi, I. and Onozaka, S. 1989). It has been reported that the stimulation of α_1 receptor which mediates GTP binding protein activates phospholipase C and results in the generation of two second messengers, inositol 1, 4, 5-triphosphate and diacylglycerol, which affect the contractile processes of vascular smooth muscle (Karaki and Weiss, 1988; Kitazawa *et al.*, 1989).

On the other hand, rat anococcygeus muscle was picked up as experimental preparation by Gillespie (1972) and it is known that this muscle is consists of a thin sheet of parallel smooth muscle fibers and dose not show a muscle tonus and spontaneous contractions but contractions induced by both adrenergic and cholinergic agonists (Gillespie, 1972; Gillespie and Lullmann-Rauch, 1974; Gillespie, 1980). As rat aorta is not affected by cholinergic agonists, it can be said that the pharmacological characteristic of anococcygeus muscle is different from that of aorta. For α_1 agonists, norepinephrine and phenylephrine (PE), induced contractions in the anococcygeus muscle and aorta, and the sensitivity of anococcygeus muscle to norepinephrine was lower than that of aorta, but those of both muscles to PE were almost similar (Shimizu *et al.*, 1992).

In this study, the effects of PE on contractility were compared between aorta and anococcygeus muscle of rat by measuring changes in muscle contraction and $[Ca^{2+}]_i$ level in intact smooth muscles and in muscle tension of permeabilized muscle preparations.

Materials and methods

Preparations

Male rats (Wistar strain; 250~300 g) were killed by a blow on the head and bled to death. After exsanguination, the thorax and abdomen were opened, the thoracic aorta and anococcygeus muscle were removed. The preparation of anococcygeus muscle which was made by the Gillespie's method (1972) was 10 mm in length and 5 mm in width. The thoracic aorta was cut into a spiral and endothelium was removed by gently rubbing the intimal surface with moistened cotton.

Simultaneous measurement of muscle tension and $[Ca^{2+}]_i$ level

As described previously (Ozaki *et al.*, 1987), muscle strips were treated with fura 2/AM (5 μM) for 3 to 4 h at room temperature. The non-cytotoxic detergent, cremophor EL (0.02%), was also added to increase the solubility of fura 2/AM. The muscle strip was hold horizontally in 8 ml volume organ bath with the physiological salt solution (PSS). One end of the muscle strip was bound with silk thread to monitor the mechanical activity, and the other end of the muscle is pinned on the bottom of the organ bath. The muscle strip was alternately excited at 340 nm and 380 nm lights through the rotating filter wheel, and 500 nm emission was measured with a fluorimeter (CAF-100, Japan Spectroscopic CO., Ltd., Tokyo, Japan). The PSS employed was a modified Tyrode's solution of the following composition (mM); NaCl,

136.8 ; KCl, 5.4 ; $CaCl_2$, 1.5 ; $MgCl_2$, 1.0 ; $NaHCO_3$, 11.9 and glucose 5.5. High K^+ solution was made by substituting NaCl with equimolar KCl. Ca^{2+}-free PSS was made by adding 0.5 mM EGTA instead of 1.5 mM $CaCl_2$. These solutions were aerated with 95% O_2, 5% CO_2 gas mixture at 37°C and pH 7.2.

Measurement of contraction in a permeabilized smooth muscle

Preparations (5.0 mm in length and 0.2 mm in width) were made from isolated rat anococcygeus muscle as a foresaid method. The muscle strip was previously hold horizontally in 1 ml volume of organ bath. One end of each strip was fixed and the other end was connected to a straingauge transducer with silk thread. The muscle contraction was isometrically recorded. This muscle was made by treating the isolated tissue with relaxing solution (RS) which contained β-escin (40 μM) for 20 to 30 minutes at room temperature. RS contained ; propionic acid potassium salt, 130 mM ; $MgCl_2$, 4 mM ; Na_2-ATP, 4 mM ; tris-maleate, 20 mM ; creatine phosphate, 2 mM ; creatine phosphokinase, 10 U/ml and EGTA, 2 mM. Each solution was adjusted to pH 6.8 at 24°C with KOH. Then, the experiment was started after exchanging a RS containing β-escin for normal RS. An addition of drug was exchanged a normal RS with a RS containing drugs. We tried to permeabilize rat aotra strip with β-escin, however it was almost impossible.

Chemicals

Chemicals used were phenylephrine, verapamil, nicardipine, β-escin, nitroprusside Na, ethyleneglycol bis (β-aminoethylether)-N, N, N′, N′-tetra-acetic acid (EGTA) (Sigma Chemical Co, St. Louis, MO), 12-deoxyphorbol 13-isobutyrate (DPB), phorbol 12-myristate 13-acetate (Funakoshi, Tokyo, Japan), 1-(5-isoquinolinesulfonyl)-2-methyl piperazine di-hydrochrolide (H-7) (Seikagaku Kogyo, Tokyo, Japan), fura 2/AM (Dojindo Laboratories, Kumamoto, Japan) and cremophor EL (Nakarai Chemical, Japan).

Statistics

Values were expressed as mean±S.E.M., and statistical analyses were performed by Student's t-test. Statistical analyses of the relationships between $[Ca^{2+}]_i$ level and muscle tension in the PE- and high K^+-treatments were done by comparison of two regression slopes.

Results

Relationships between muscle tension and $[Ca^{2+}]_i$ level in aorta and anococcygeus muscle

Effects of PE and high K^+ were examined in the simultaneous measurement of muscle tension and $[Ca^{2+}]_i$ level using fura 2 method in aorta and anococcygeus muscle. In aorta and anococcygeus muscle, an application of 1 μM PE induced an elevation of $[Ca^{2+}]_i$ level followed by a contraction, after that the increases in $[Ca^{2+}]_i$ level and muscle tension were sustained at high levels (Fig. 1). Moreover, an application of isosmotic 77 mM KCl solution (Iso-77K^+) also induced a sustained increase in $[Ca^{2+}]_i$ level and muscle tension in aorta and anococcygeus muscle (Fig. 2). A cumulative application of various concentrations of PE or isosmotic high K^+

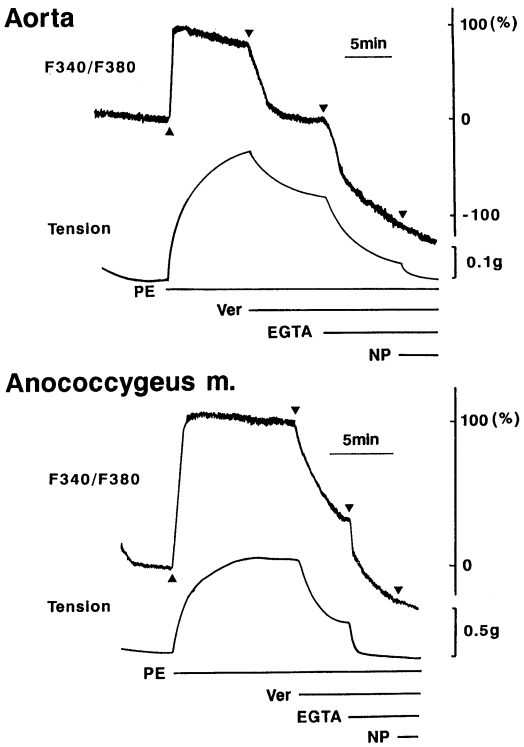

Fig. 1. Effects of verapamil on $[Ca^{2+}]_i$ level (upper trace) and muscle tension (lower trace) in rat aorta or anococcygeus muscle stimulated by phenylephrine (PE: 1 μM).
Antagonists were sequentially applied. Ver: 10 μM verapamil. EGTA: 4 mM EGTA. NP: 1 μM nitroprusside. 100% represents the PE-stimulated $[Ca^{2+}]_i$ level measured previously in the same muscle strip.

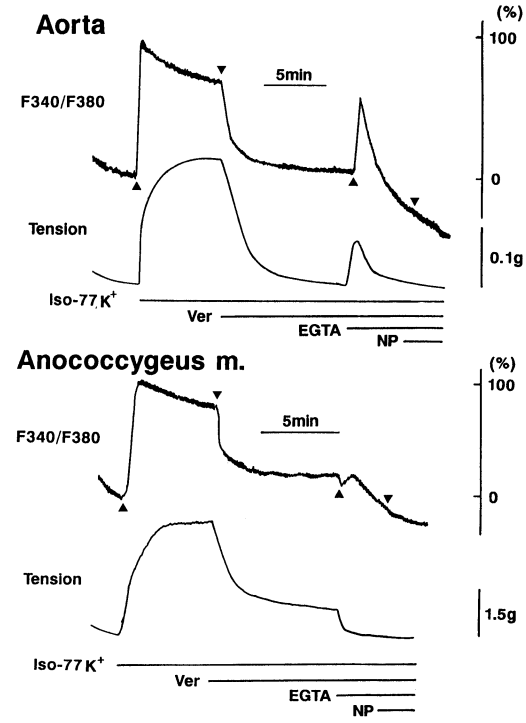

Fig. 2. Effects of verapamil on $[Ca^{2+}]_i$ level (upper trace) and muscle tension (lower trace) in rat aorta or anococcygeus muscle stimulated by 77 mM KCl (Iso-77K$^+$).
Antagonists were sequentially applied. Ver: 10 μM verapamil. EGTA: 4 mM EGTA. NP: 1 μM nitroprusside. 100% represents Iso-77K$^+$-stimulated $[Ca^{2+}]_i$ level measured previously in the same muscle strip.

solution (Iso-K$^+$) induced dose-dependent increases in muscle tension and $[Ca^{2+}]_i$ level in aorta. These correlations were positive and showed $y=1.7x-31.2$ ($r=0.99$) in the PE-treatment and $y=1.2x-34.7$ ($r=0.97$) in the Iso-K$^+$ one, respectively (Fig. 3).

A cumulative application of PE or Iso-K$^+$ to anococcygeus muscle also induced a dose-dependent increases in $[Ca^{2+}]_i$ level and muscle tension. The correlations in the PE- or Iso-K$^+$-treatment in anococcygeus muscle were positive as similar to that in aorta and showed $y=1.4x-29.5$ ($r=0.98$) and $y=1.2x-25.8$ ($r=0.98$), respectively (Fig. 3). Comparing the relationships between $[Ca^{2+}]_i$ level and muscle tension in the PE- and high K$^+$-treatments, PE induced a larger contraction than high K$^+$ at a given $[Ca^{2+}]_i$ in the aorta. The difference of regression slopes in the relationship between $[Ca^{2+}]_i$ level and muscle tension under PE- and high K$^+$-treatments in aorta was significant, but that in anococcygeus muscle is not (Fig. 3). These results indicate that there is a difference between Ca^{2+} sensitivity to PE and high K$^+$ only in the aorta.

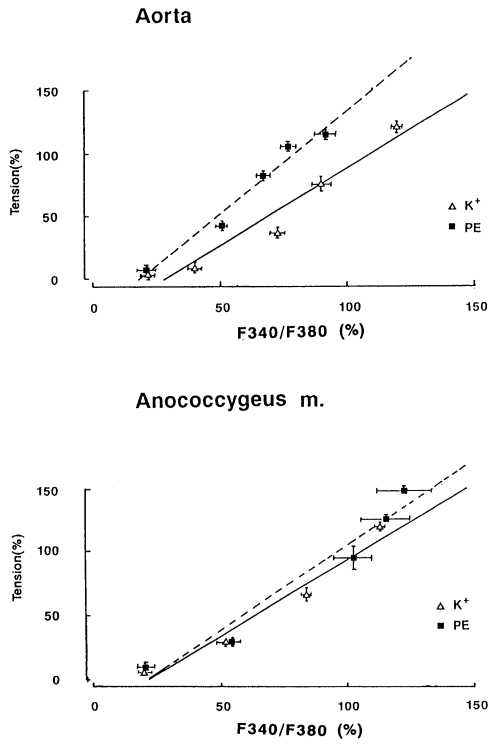

Fig. 3. $[Ca^{2+}]_i$-tension relationship in rat aorta and anococcygeus muscle in the presence of isosmotic high $K^+(K^t)$ or phenylephrine (PE).
Each point represents mean ±S.E.M. of 6 ~8 experiments. The $[Ca^{2+}]_i$ level and muscle tension are shown by relative values taking those in the presence of Iso-77K^+ as 100%.

Effects of Ca^{2+} antagonists on the PE-induced contraction

Effects of Ca^{2+} antagonists, verapamil and nicardipine was examined on the PE-induced contraction in the muscles. An application of 10 μM verapamil to the precontracted muscle by PE (1 μM) decreased the developed tension to 62.0% and elevated $[Ca^{2+}]_i$ level to 3.8% in aorta, respectively (Fig. 1, Table 1). Nicardipine (3 μM) showed similar effects on both the parameters to those by verapamil (data not shown). A further addition of 4 mM EGTA to exclude an

Table 1. Effects of verapamil, EGTA and nitroprusside on $[Ca^{2+}]_i$ level and muscle tension in rat aorta or anococcygeus muscle stimulated with 1 μM phenylephrine (PE).
Each value represents the mean (with s.e.mean) of 6 experiments. 100% represents $[Ca^{2+}]_i$ level and muscle tension stimulated by 1 μM PE.

	Aorta		Anococcygeus m.	
	Tension (%)	$[Ca^{2+}]_i$ level (%)	Tension (%)	$[Ca^{2+}]_i$ level
PE (1 μM)	100	100	100	100
+Verapamil (10μM)	62.0±3.7	−3.8±18.0	42.1±4.2	34.3±5.1
+Verapamil + EGTA (4 mM)	18.3±3.4	−145.5±53.9	0	−21.0±5.1
+Verapamil + EGTA + Nitroprusside (1 μM)	0	−197.1±60.8	0	−35.9±5.8

extracellular Ca^{2+} decreased the $[Ca^{2+}]_i$ level much below the resting one, but partially remained the developed tension. A sequential application of 1 μM nitroprusside completely abolished the residual contraction and further decreased $[Ca^{2+}]_i$ level.

In anococcygeus muscle, 1 μM PE induced sustained increases in contractile tension and $[Ca^{2+}]_i$ level. An application of 10 μM verapamil partially reduced the increase in muscle tension and $[Ca^{2+}]_i$ level to 42.1% and 34.3%, respectively. EGTA (4 mM) decreased the $[Ca^{2+}]_i$ level below the resting one, but kept muscle tension at the resting level. Nitroprusside less decreased the $[Ca^{2+}]_i$ level in anococcygeus muscle than that in aorta (Fig. 1, Table 1). Nicardipine (3 μM) showed similar effects to those of verapamil.

Effects of Ca^{2+} antagonists on high K^+-induced contraction

An application of 10 μM verapamil to the precontracted aorta by Iso-77K^+ decreased the developed tension and elevated $[Ca^{2+}]_i$ level to the resting levels, respectively. A sequential application of 4 mM EGTA induced a remrakably transient increases in both the muscle tension and $[Ca^{2+}]_i$ level, then the tension kept at the resting level and $[Ca^{2+}]_i$ level fell down below the resting one. The decreased $[Ca^{2+}]_i$ level further fell down after an application of 1 μM nitroprusside. In the anococcygeus muscle, an application of 10 μM verapamil showed significantly decreased the increases in muscle tension and $[Ca^{2+}]_i$ level induced by Iso-77K^+, but kept both the levels above the resting ones. A sequential application of 4 mM EGTA returned the tension and $[Ca^{2+}]_i$ level to the resting one. EGTA did not show transient increases in muscle tension and $[Ca^{2+}]_i$ level in the anococcygeus muscle, as shown in aorta. The addition of 1 μM nitroprusside further decreased $[Ca^{2+}]_i$ level below the resting one (Fig. 2).

Effects of phorbol ester (DPB) on muscle tension and $[Ca^{2+}]_i$ level

Effects of phorbol ester, 12-deoxyphorbol 13-isobutyrate (DPB) on muscle tension and $[Ca^{2+}]_i$ level in each muscle was examined. An application of Iso-77K^+ markedly induced a sustained increase of $[Ca^{2+}]_i$ level and muscle tension in the aorta. After a removal of Iso-77K^+, an application of 1 μM DPB induced the increase in tension at approximately 150% of Iso-77K^+-developed tension and $[Ca^{2+}]_i$ level at approximately 50% of Iso-77K^+-evelated level. However, application of DPB had no effect on muscle tension and $[Ca^{2+}]_i$ level in the anococcygeus muscle (Fig. 4). On the other hand, in the Ca^{2+}-free medium containing 0.5 mM EGTA, DPB had no effects on $[Ca^{2+}]_i$ level while it increased a tension which was similar to that by Iso-77K^+ in the aorta. However, DPB had also no effect on both the tension and $[Ca^{2+}]_i$ in Ca^{2+}-free medium in the anococcygeus muscle. These data was not shown in figure.

Transient increases in tension and $[Ca^{2+}]_i$ level induced by PE in Ca^{2+}-free medium

After Iso-77K^+ induced a contraction of aorta in the normal PSS, an application of 10 μM PE induced a transient increase in muscle tension and $[Ca^{2+}]_i$ level in anococcygeus muscle treated with a Ca^{2+}-free medium with 0.5 mM EGTA for 5 min. A PE-induced transient contraction was 92.7$\pm$2.9% of the sustained contraction induced by Iso-77K^+, and a PE-elevated $[Ca^{2+}]_i$ level was 112.2$\pm$6.5% of that elevated by Iso-77K^+. In anococcygeus muscle

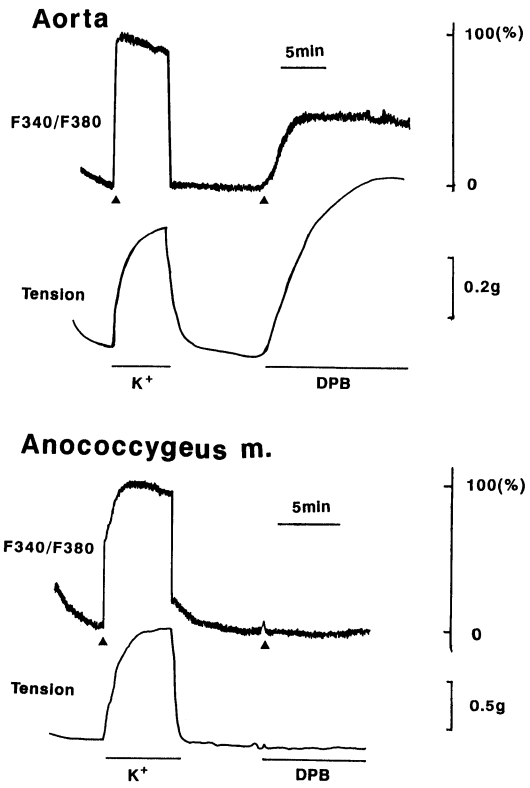

Fig. 4. Effects of DPB (1 μM) on $[Ca^{2+}]_i$ level (upper trace) and muscle tension (lower trace in aorta and anococcygeus muscle of rat.
F340/F380 is taking a resting level as 0% and the Iso-77K$^+$(K^+)-stimulated level as 100%.

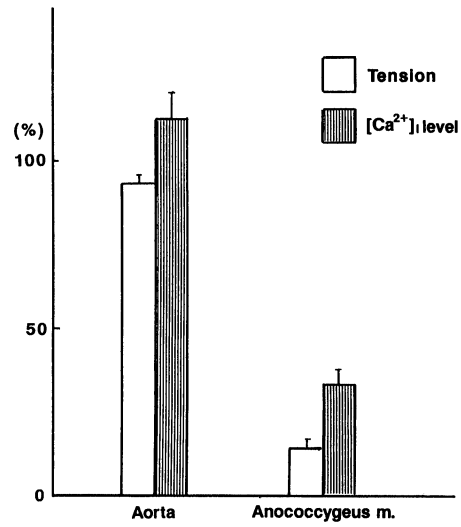

Fig. 5. Transient increases by PE in $[Ca^{2+}]_i$ level and muscle tension in Ca^{2+}-free solution. Aorta or anococcygeus muscle strip was stimulated by Iso-77K$^+$ followed by a wash with Ca^{2+}-free solution. Five min later, 10 μM PE was added. 100% represents the Iso-77K$^+$-stimulated $[Ca^{2+}]_i$ level and muscle tension measured previously in the same muscle strip. Each point represents the mean $\pm$S.E.M. of 6 experiments.

soaked in Ca^{2+}-free medium, an application of PE induced small transient increments of muscle tension and $[Ca^{2+}]_i$ level, and the increase of tension by PE was 14.6$\pm$3.3% that by Iso-77K$^+$ and the elevated $[Ca^{2+}]_i$ level by PE was 33.3$\pm$6.2% of that by Iso-77K$^+$. It was indicated that the PE-induced transient contraction which was mediated by Ca^{2+} release from an intracellular storage in anococcygeus muscle is much smaller than that of aorta (Fig. 5).

Ca^{2+}-developed tension in permeabilized muscle of anococcygeus muscle

In rat anococcygeus muscle made permeable by β-escin, an addition of 3 μM Ca^{2+} developed tension which reached a maximal level within 10 min and kept steady. PE (10 μM) with 10 μM GTP shifted the pCa-tension curve for Ca^{2+} to the left at range of Ca^{2+} concentration from 0.3 μM to 3 μM in the rat anococcygeus muscle (Fig. 6). GTPγS (10 μM), a non-hydrolyzable analogue of GTP, also shifted the pCa-tension curve for Ca^{2+} to the left at Ca^{2+}

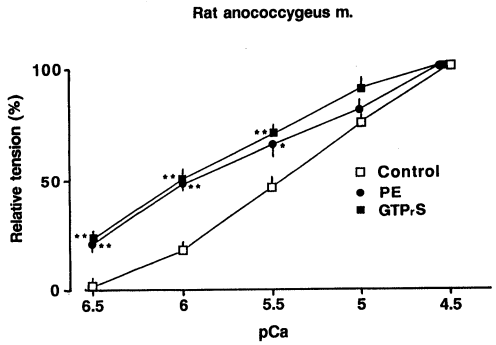

Fig. 6. Ca^{2+}-tension relationship in permeabilized rat anococcygeus muscle.
Ca^{2+} was cumulatively added in the absence (control, □) or presence of phenylephrine (10 μM) with GTP (10 μM) (●) or GTPγS (10 μM) (■). Each point is the mean value derived from 6 experiments and vertical bars represent the S.E.M. * and **, significant difference from control (*$P<0.05$, **$P<0.01$).

concentration from 0.3 μM to 30 μM.

Discussion

Muscle tension has been simultaneously measured with $[Ca^{2+}]_i$ level using the fura 2 method in vascular smooth muscle of rat. The measurement has revealed that sustained contractions induced by high K^+ and receptor agonists are due to an elevation of $[Ca^{2+}]_i$ level from resting one (Ozaki *et al.*, 1987; Sato *et al.*, 1988; Sakata *et al.*, 1989; Ozaki *et al.*, 1990; Thorin-Threscases *et al.*, 1990; Mitsui *et al.*, 1993). High K^+ and agonists induced a concentration-dependent increase in both the $[Ca^{2+}]_i$ level and muscle tension in aorta, with a good correlation between these two parameters. The $[Ca^{2+}]_i$-tension relationship in the presence of high K^+ or agonist evidenced that agonists induced a greater tension at the same amount of $[Ca^{2+}]_i$ than high K^+ (Karaki *et al.*, 1988; Sato *et al.*, 1988; Karaki, 1989). In the present experiment, the relationship between $[Ca^{2+}]_i$ and muscle tension in aotra and anococcygeus muscle by applying high K^+ or PE indicated a positive correlation. PE induced a larger contraction than high K^+ at a given $[Ca^{2+}]_i$ in the aorta. Moreover, a difference of regression slopes in the relationships between $[Ca^{2+}]_i$ level and muscle tension under PE- and high K^+-treatments in aorta were significant. The result suggests that a Ca^{2+} sensitization of contractile element to PE seen in aorta, but not in anococcygeus muscle.

It was reported that Ca^{2+} blockers such as verapamil or nicardipine decreased $[Ca^{2+}]_i$ to a resting level from one elevated by norepinephrine in rat aorta, though the blockers partially decreased the developed tension by the agonist. When an extracellular Ca^{2+} was removed by the sequential treatment with EGTA, the $[Ca^{2+}]_i$ level decreased below the resting one, but the contractile tension still remained at a certain level (Ozaki *et al.*, 1990). As described before, effects of the Ca^{2+} antagonists and EGTA on the PE-induced contraction in aorta was similar to those on the norepinephrine-induced contraction. However, in the anococcygeus muscle precontracted by PE, the verapamil-treatment decreased together the elevated $[Ca^{2+}]_i$ level and developed tension to about 40% of control. And EGTA decreased $[Ca^{2+}]_i$ below the resting level, and returned the muscle tension to the resting level. Therefore, anococcygeus muscle did not show the partial residue of the developed tension at the resting level of $[Ca^{2+}]_i$, as aorta did. On the other hand, verapamil decreased the elevated $[Ca^{2+}]_i$ level and developed

tension by high K^+ almost to the resting level in both the aorta and anococcygeus muscle. The results suggest that a contraction induced by PE or high K^+ is mainly due to Ca^{2+} influx in both the muscles. Moreover, the PE-induced contraction seems to be involved with other two factors, one is an enhancement of Ca^{2+} sensitivity to PE and another is a contractile mechanism which is independent of Ca^{2+}. The latter has been reported in the NE-induced contraction of rat aorta (Karaki, 1990). These two factors may less participate to anococcygeus muscle than aorta. Furthermore, the decline in $[Ca^{2+}]_i$ level by EGTA was more sharp and deeper in aorta than in anococcygeus muscle. This result suggests that aorta shows a larger difference between the zero level of $[Ca^{2+}]_i$ and the resting level of $[Ca^{2+}]_i$, which means a larger retained amount of $[Ca^{2+}]_i$ than anococcygeus muscle.

In a Ca^{2+}-free medium, PE induced a rapidly transient increase in $[Ca^{2+}]_i$ level in aorta, then reduced it below the resting one. PE also elicited a large transient contractions followed by small sustained one in aorta. Application of high K^+ did not cause any change in either $[Ca^{2+}]_i$ level or muscle tension in aorta soaked in Ca^{2+}-free medium. On the other hand, there was an extremely small increase in both the parameters when anococcygeus muscles were treated with PE, indicating a quitely different result from that of the aorta. As sarcoplasmic reticulum (SR) in anococcygeus muscle was reported to be morphologically underdeveloped one (Gillespie and Lullmann-Rauch, 1974), there would be less Ca^{2+} release from the SR when the muscle is treated with PE in Ca^{2+}-free medium. Moreover, an application of verapamil to the precontracted aorta by high K^+ decreased $[Ca^{2+}]_i$ level and muscle tension, then the sequential addition of EGTA remarkably induced a transient increase in both of $[Ca^{2+}]_i$ level and muscle tension before showing the sharp decline of $[Ca^{2+}]_i$ level. On the other hand, in anococcygeus muscle, EGTA decreased the $[Ca^{2+}]_i$ level and muscle tension without transiently increased changes. This finding also supports that SR in anococcygeus muscle is underdeveloped one. From these results, it is suggested that the contraction by PE in rat anococcygeus muscle are mainly dependent on Ca^{2+} influx and less dependent on Ca^{2+} release from intracellular storage.

DPB, a phorbol ester that activates protein kinase C, produced a large contraction and significant increase in $[Ca^{2+}]_i$ level and the DPB-induced contraction is insensitive to EGTA in Ca^{2+}-free medium (Karaki *et al.*, 1991). However, the $[Ca^{2+}]_i$ level and muscle tension was not changed by DPB treatment on the anococcygeus muscle, which was probably due to an organ difference in phorbol ester-effect, as reported by Karaki (1990). Thus, anococcygeus muscle may be less involved with a contractile mechanism that is activated by protein kinase C as seen in aorta.

Skinning of smooth muscle using α-toxin of bacteria recently has been developed to evaluate the change in Ca^{2+} sensitivity by measuring the muscle tension at a certain $[Ca^{2+}]_i$ level (Nishimura *et al.*, 1988 ; Kitazawa *et al.*, 1989). It was noted that an agonist enhanced a Ca^{2+}-induced tension in the presence of GTP in the experiment using the α-toxin-skinned preparation (Kitazawa *et al.*, 1989). A similar result was obtained by GTPγS which activates GTP-binding protein and is not hydrolized (Kitazawa *et al.*, 1989 ; Nishimura *et al.*, 1990). A β-escin-treated skinned fiber, prepared by making a small hole in the cell membrane to maintain an intracellular second messenger system, can also be used in the experiment in the same way as α-toxin (Kobayashi *et al.*, 1989). The Ca^{2+}-developed tension in the β-escin-

skinned preparation of anococcygeus muscle was enhanced by PE in the presence of GTP, as well as with GTPγS. A stimulation of the receptor with PE is considered to increase the Ca^{2+} sensitivity of contractile elements by mediating GTP-binding protein in the anococcygeus muscle.

In summary, the contraction of rat anococcygeus muscle by the α_1 agonists considered to be mainly dependent on Ca^{2+} influx regulated by receptor, and less an Ca^{2+} release from on intracellular storage. However, the enhancement of Ca^{2+} sensitization to PE may be significantly less and the Ca^{2+} independent contractile mechanism would be absent in anococcygeus muscle, being differense in those in aorta.

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