Contractile Response of Spontaneously Hypertensive Rat Caudal Artery to Phorbol Esters

LUSIANE M. BENDHACK, RAM V. SHARMA, AND RAMESH C. BHALLA

SUMMARY Stimulation of phosphatidylinositol metabolism by neurotransmitters produces diacylglycerol, an activator of protein kinase C, which may be involved in hormone-mediated contractions. We studied the effect of a tumor-promoting phorbol ester, 12-deoxyphorbol 13-isobutyrate 20-acetate (DPBA), on contraction of caudal artery rings of Wistar-Kyoto control (WKY) and spontaneously hypertensive rats (SHR) in order to examine whether protein kinase C-mediated mechanisms are increased in SHR. Although DPBA alone did not produce contractions of either WKY or SHR caudal artery rings, it greatly potentiated the contractions evoked by norepinephrine, vasopressin, potassium, and calcium ionophore A23187. The potentiation of contractile response to these agents by DPBA was dependent on extracellular calcium. The DPBA potentiation of contractions evoked by norepinephrine, vasopressin, and potassium was significantly greater (p < 0.05) in SHR than in WKY, while no differences were observed between strains for the contractions evoked by calcium ionophore A23187. These results indicate that the protein kinase C-mediated responses are increased in SHR caudal artery rings, and this effect appears to be due to increased calcium influx through cell membrane calcium channels. (Hypertension 11 [Suppl I]: I-112–I-116, 1988)

KEY WORDS • norepinephrine • vasopressin • potassium • A23187 • calcium influx • calcium channels

It has been demonstrated that the calcium sensitivity of norepinephrine (NE)-stimulated arterial rings is increased in spontaneously hypertensive rats (SHR) as compared to Wistar-Kyoto rats (WKY; for recent review, see Reference 1). We have further observed that the increased calcium sensitivity in SHR caudal artery is due specifically to a defect in α-adrenergic receptor-mediated mechanisms distal to the NE binding to the receptors. Recently, it was noted that in vascular smooth muscle phosphoinositide metabolism is increased during hormonal stimulation and inositol 1,4,5-trisphosphate (IP3) is accumulated rapidly. Furthermore, the addition of IP3 to permeabilized vascular smooth muscle cells and arterial strips produced repeated contractions and also calcium release from intracellular stores, presumably in the sarcoplasmic reticulum (SR). In addition, it has been shown that NE stimulation results in increased intracellular calcium release in the caudal artery and in the mesenteric resistance vessels of SHR, which could be explained by an increased production of IP3, in response to α-adrenergic receptor stimulation.

It has been shown that tumor-promoting phorbol esters activate protein kinase C (for a recent review, see Reference 10) and also produce vascular smooth muscle contraction. Since vascular smooth muscle contains sufficiently large amounts of protein kinase C, it is possible that this branch of second messenger system may also be augmented in SHR to produce increased sensitivity of vascular smooth muscle to calcium. Although the precise mechanisms by which phorbol esters produce vascular smooth muscle contraction remain to be defined, it is generally accepted that they activate membrane-bound protein kinase C by increasing its affinity to calcium (see Reference 10), which may result in the phosphorylation of specific proteins responsible for vascular smooth muscle contraction. It is thus possible that in SHR caudal artery the hormone-mediated hydrolysis of membrane-bound phosphatidylinositol 4,5-biphosphate may be increased, leading to increased production of IP3 and diacylglycerol (DAG), both of which may act synergistically to produce increased contraction in SHR vascular smooth muscle.

In the present investigation we studied the possible role of DAG and protein kinase C in contractions...
mediated by NE, vasopressin, potassium, and A23187 in SHR caudal artery rings. Since phorbol esters and DAG stimulate protein kinase C by the same mechanism,16 we used tumor-promoting phorbol ester 12-deoxyphorbol 13-isobutyrate 20-acetate (DPBA) to mimic endogenously produced DAG in caudal artery rings of WKY and SHR. Our results demonstrate that preincubation of caudal artery rings with DPBA significantly augmented contractile response to NE, vasopressin, and potassium, although DPBA alone did not produce any discernible contraction. Furthermore, DPBA potentiation of contractile response of caudal artery rings stimulated with NE, vasopressin, and potassium was significantly greater in SHR than in WKY.

Materials and Methods

Male SHR and WKY rats (Taconic Farms, Germantown, NY, USA) used in these experiments were approximately 35 to 40 weeks of age. Preoperative systolic blood pressure was determined in the unanesthetized state, using an automated cuff-inflator pulse-reading system. The average blood pressure of SHR (199.3 ± 4.8 mm Hg; n = 22) was significantly higher (p < 0.01) than that of WKY rats (128.1 ± 2.2 mm Hg; n = 22). The ventral caudal artery was removed from the rats under ether anesthesia and 3-mm rings were cut from the proximal end under a dissecting microscope. The arterial rings were mounted between two stainless steel hooks in the opposite sides of the wall of the artery to measure tension from the circumferential axis of the vessel wall, which was recorded using a Beckman R611 multichannel recorder (Beckman Instruments, Fullerton, CA, USA). The arterial rings were denervated using 6-OH dopamine as described earlier.2 In all the experiments described, denervated arterial rings were used. Resting tension was adjusted to 2 g, which was found to produce maximum active tension in response to hormones and potassium.2 For 3 hours before initiating the experiment, arterial rings were allowed to equilibrate in oxygenated physiological salt solution of the following composition (mM): NaCl, 130; KCl, 4.7; NaHCO3, 14.9; NaH2PO4, 1.18; MgSO4, 1.17; dextrose, 5.5; CaCl2, 1.6; EDTA, 0.027; HEPES, 13 (pH 7.4). At the beginning of the experiment, caudal artery rings were repeatedly stimulated with the given vasoactive agent until reproducible contractions were obtained. Then the effects of DPBA alone and in combination with vasoactive agents were measured on the same arterial rings.

DPBA (LC Services, Woburn, MA, USA) was dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 10 mM. DPBA was used in place of more commonly used phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) because it is slightly more water-soluble. The final concentration of DMSO in the muscle bath was never more than 0.1%, and at this concentration DMSO per se did not significantly affect basal or hormone-stimulated response. Results are expressed as maximum isometric tension (N × 10⁻³/m²). Surface area was calculated as described earlier.2 Statistical analysis was done using Student's t test, and differences were considered significant at a p value below 0.05.

Results

A potent phorbol ester DPBA (0.1–1 μM) when added alone did not evoke contractions in caudal artery rings after up to 60 minutes of incubation. However, 30–45-minute preincubation with 1 μM DPBA produced rapid potentiation of NE-mediated contraction (Figure 1, left panel). Both NE alone and NE plus DPBA produced significantly greater (p < 0.05) contraction in caudal artery of SHR than in that of WKY. Furthermore, the net DPBA potentiation was approximately 75% greater in SHR than in WKY (Table 1).
To determine whether increased DPBA potentiation of caudal artery contraction in SHR is specific to α1-adrenergic receptors or occurs in response to other contractile hormones, we tested the effect of DPBA on lysine vasopressin–mediated contraction (see Figure 1, right panel). DPBA greatly potentiated the contractile response of caudal artery rings to vasopressin, and the potentiation was approximately two times greater in SHR than in WKY (see Table 1). In addition, vasopressin (2 mU/ml) produced significantly greater (p < 0.05) contraction in SHR caudal artery rings than in those of WKY. Similarly, vasopressin in calcium-free medium also produced significantly greater (p < 0.05) contraction of SHR caudal artery rings (0.58 ± 0.06 N × 10⁷ m²; n = 14) compared to WKY (0.38 ± 0.04 N × 10⁷ m²; n = 14).

We showed earlier that neither potassium-induced contraction nor potassium-induced calcium influx is significantly different in WKY and SHR caudal artery rings. Therefore, it was of interest to study whether DPBA would potentiate the contractile responses to potassium differentially between WKY and SHR caudal artery rings. In these experiments, we examined the effect of 1 μM DPBA on potassium-induced contractions of WKY and SHR caudal artery rings. A 45-minute preincubation of caudal artery rings greatly potentiated the contractions evoked by 60 mM potassium in both WKY and SHR. (Figure 2, left panel). Again, the net DPBA-mediated potentiation was approximately 85% more in SHR than in WKY (see Table 1). On the other hand, the maximum contractions evoked by potassium alone were not significantly different between the two strains.

We further tested whether increased potentiation by DPBA of hormone- and potassium-stimulated contraction is due to its effect on calcium channels or on the contractile response:
contractile proteins by examining the effects of DPBA on A23187-stimulated contractions of WKY and SHR caudal artery rings (see Figure 2, right panel). Pretreatment of caudal artery rings with 4 μM A23187 for 10 to 20 minutes in calcium-free media produced comparable contractions between WKY and SHR caudal artery rings when subsequently exposed to physiological salt solution containing 1.6 mM calcium. Furthermore, addition of DPBA together with calcium to A23187-pretreated arterial rings significantly (p<0.05) potentiated the contractile response in both WKY and SHR (see Figure 2, right panel). However, the net DPBA potentiation was not significantly different between strains.

Potentiation by DPBA of hormone-, potassium-, and A23187-stimulated contraction was dependent on extracellular calcium and could be reversed by removing calcium from the bathing medium in both WKY and SHR caudal artery rings (see Figure 1, left panel; Figure 2, right panel). Also, DPBA potentiation of contractile response to all the contractile agents was reversible upon removing the contractile agent and DPBA, and washing the tissue with physiological salt solution containing 1.6 mM calcium in both WKY and SHR caudal artery rings (see Figure 1, right panel; Figure 2, left panel). These results indicate that the DPBA-mediated contractions are dependent on calcium influx and the continued presence of contractile agent in both WKY and SHR caudal artery rings is necessary.

Discussion

The present results indicate that in addition to increased α1-adrenergic receptor–stimulated contractions observed earlier,1,4,7 lysine vasopressin–stimulated contractions are also increased in SHR caudal artery rings compared to WKY rings in the presence and absence of extracellular calcium. Since NE and vasopressin have been shown to stimulate phosphatidylinositol turnover in vascular smooth muscle,18 it is possible that hormone-stimulated production of IP3 and DAG is increased in SHR caudal artery rings. Increased production of IP3 would result in increased calcium release from SR and be responsible, at least in part, for increased contractions observed in calcium-free solution in response to NE5 and also in response to vasopressin (see Results).

Augmented hormone-stimulated turnover of phosphoinositides in SHR also results in increased production of DAG, which, in turn, results in increased activation of protein kinase C. Thus, if tumor-promoting phorbol esters mimic DAG,10,16 one would expect them to produce greater contraction in SHR caudal artery rings similar to that observed with hormones. Our major findings are that DPBA significantly potentiated the contraction of caudal artery rings stimulated with NE, vasopressin, potassium, and A23187 (see Table 1), and that DPBA-mediated potentiation of contractile responses evoked by NE, vasopressin, and potassium were significantly increased in SHR caudal artery rings compared with WKY rings. These results suggest that protein kinase C plays a physiological role on caudal artery contraction and that C-kinase–mediated mechanisms may be increased in SHR.

Since phorbol esters alone did not produce the contraction of caudal artery rings but potentiated the contractile responses evoked by hormones, potassium, and A23187, it would appear that they increase the calcium sensitivity of protein kinase C, resulting in an increased activity of this enzyme. These results support the hypothesis of Nishizuka,10 suggesting that phorbol esters increase the affinity of protein kinase C for calcium. Since increased NE-stimulated calcium influx as well as calcium release from SR has been demonstrated in SHR vascular smooth muscle,4,7 it is expected that the intracellular calcium concentration will be increased in SHR. Similarly, it has been demonstrated that vasopressin-stimulated intracellular calcium concentration is increased in SHR vascular smooth muscle.8 Therefore, the increased augmentation of NE- and vasopressin-mediated contractions by DPBA in SHR caudal artery may reflect a synergistic interaction between increased intracellular calcium levels due to hormonal stimulation and DPBA to produce increased protein kinase C activation. Furthermore, the increased activation of protein kinase C in SHR caudal artery rings may then lead to increased phosphorylation of membrane proteins, resulting in further increase in calcium influx through cell membrane calcium channels. Similar to our observations, Baraban et al.12 showed that phorbol esters reversibly augment the contractile responses to hormones and calcium in basilar artery and vas deferens smooth muscle.

In addition to greater potentiation of hormone-stimulated contraction, DPBA potentiated the contractile response to potassium to a greater extent in SHR than in WKY caudal artery rings. Unlike hormones, however, potassium-stimulated contraction and calcium influx were not significantly different between WKY and SHR. These data therefore indicate that DPBA-mediated activation of protein kinase C may result in increased calcium influx in SHR caudal artery through potential-dependent calcium channels. Wei and Triggle11 showed that TPA greatly potentiated the contractile responses of rat caudal artery to potassium and calcium channel agonist Bay k 8644. In addition, potassium and Bay k 8644 have been shown to decrease the latency period for the activation of rabbit ear artery contractions by TPA.11 In view of these observations, it is possible that increased calcium influx in response to phorbol esters may be due to activation of the potential-dependent calcium channels by protein kinase C–dependent phosphorylation.

In our study as well as those of Wei and Triggle,13 phorbol esters augmented the contraction of caudal artery rings only in the presence of potassium or Bay k 8644. Thus it seems likely that calcium channel phosphorylation by phorbol esters increases the calcium conductance after channels are activated by depolarization or by calcium channel agonists. A direct measurement of unidirectional calcium influx in WKY
and SHR caudal arteries after DPBA treatment will be helpful to elucidate the mechanism of action of protein kinase C. Alternatively, it is possible that either protein kinase C levels or its substrates may be altered in SHR, thus resulting in greater potentiation of contractile responses of caudal artery rings to hormones and potassium. This interpretation is unlikely, however, since A23187-mediated contractions, which bypass hormone receptors and potential-dependent channels, were potentiated to a similar extent in WKY and SHR by DPBA. One possible interpretation of these results is that, through the activation of protein kinase C, DPBA has a direct effect on calcium influx and thus may act synergistically with potassium and hormones to increase calcium influx markedly through cell membrane calcium channels. This action of DPBA seems to be increased in SHR and may be responsible for increased potentiation of hormones and potassium-stimulated contraction of SHR caudal artery compared to WKY.

Since our manuscript was submitted, another paper has appeared showing increased sensitivity of mesenteric artery strips of stroke-prone SHR to phorbol esters. Unlike this study, however, our results show that phorbol esters alone do not produce contraction of caudal artery rings. Similarly, Wei and Triggle showed that phorbol esters alone do not produce contraction in the rat caudal artery, but they do evoke significant contraction in rat aorta. These results indicate that effects of phorbol esters alone differ in the different arteries.

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References
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L M Bendhack, R V Sharma and R C Bhalla

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