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PROTEIN KINASE C POTENTIATES STRETCH-INDUCED CEREBRAL ARTERY TONE BY INCREASING INTRACELLULAR SENSITIVITY TO Ca^{2+} [1]

Ismail Laher, Peter Vorkapic*, Amy L. Dowd and John A. Bevan

Department of Pharmacology and Vermont Center for Vascular Research
College of Medicine
University of Vermont
Burlington, Vermont 05405

*Department of Neurosurgery
University of Vienna
Medical School
A-1090 Vienna
Austria

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The effects of PMA, an activator of protein kinase C, was studied on Ca^{2+}-induced tone in the rabbit basilar artery. Contractile responses to Ca^{2+} occurred only in arteries pretreated with PMA; the extent of Ca^{2+}-induced contractions were related to the level of stretch applied to the vessels. Bay K 8644, a Ca^{2+}-channel agonist, at a concentration that was subthreshold for contraction, augmented the extent of Ca^{2+}-induced tone occurring in PMA-treated arteries. Nifedipine, a Ca^{2+}-entry inhibitor, and staurosporine, an inhibitor of protein kinase C attenuated the response to Ca^{2+} occurring either in the absence or presence of Bay K 8644. Our results suggest that PMA increases myofilament sensitivity to Ca^{2+}, such that levels of Ca^{2+} previously ineffective for contraction Ca^{2+}-influx, e.g. due to Bay K 8644, is manifest as contraction. Our results also confirm the role of extracellular Ca^{2+} entry via plasma membrane stretch-dependent Ca^{2+}-channels in the maintenance of vascular tone in the basilar artery.

Vascular smooth muscle activation, for example by neurotransmitter agonists, is accompanied by phospholipid breakdown leading to the formation of a number of products (1). One of these is inositol 1,4,5 triphosphate, a water soluble substance capable of releasing Ca^{2+} from the sarcoplasmic reticulum of arteries (2). Another key product formed in response to membrane stimulation is diacylglycerol, a lipid soluble activator of protein kinase C (3). Under in vitro conditions, protein kinase C is permanently activated by

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Abbreviations
PMA - phorbol 12-myristate 13-acetate; PSS - physiological salt solution.

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compounds such as PMA (4). Activation of protein kinase C by either diacylglycerol or PMA causes conversion of protein kinase C from a Ca^{2+}-insensitive to Ca^{2+}-sensitive form (5). This results in the enzyme being fully active at resting levels of intracellular Ca^{2+} (1,6). Activated protein kinase C and intracellular Ca^{2+} have been proposed to act synergistically to initiate many physiological responses, including an increase in vascular tone (7,8). In this regard, a number of studies have indicated that α-adrenoceptor agonists produce maintained vascular tone possibly by altering the force-intracellular Ca^{2+} relationship. Thus upon receptor stimulation, greater force is possible at lower levels of free ca^{2+} (9,10). Evidence for protein kinase C mediated increased intracellular sensitivity to Ca^{2+} has been obtained in detergent-skinned coronary artery (11) and more recently in α-toxin skinned mesenteric resistance-artery preparations (12).

In this study we examine the role of protein kinase C activation in regulation of tone due to Ca^{2+}-entry occurring via stretch-induced mechanisms in the unskinned rabbit basilar artery, under circumstances when the intracellular milieu and resting membrane potential is preserved. Evidence for a unique regulation of stretch-induced myogenic tone by protein kinase C has been obtained in the rabbit facial vein, where stretch-induced tone is augmented by PMA (13) and attenuated by staurosporine, an inhibitor of protein kinase C (14). In PMA-pretreated rabbit basilar artery segments, the extent of Ca^{2+}-channel agonist Bay K 8644, at concentrations that were subthreshold for stretch-induced contraction augmented the extent of Ca^{2+}-induced tone in PMA treated segments. We propose that upon activation of protein kinase C, the myofilament sensitivity to Ca^{2+} is increased such that the augmented Ca^{2+}-influx upon stretch due to Bay K 8644 is manifest in maintained tone.

METHODS: Experiments were made using basilar arteries isolated from male rabbits weighing 3 to 4 kg. The basilar artery was dissected free in PSS of the following composition (in millimolar): Na^+, 144.2; K^+, 4.9; Ca^{2+}, 1.6; Mg^{2+}, 1.2; Cl^-, 126.7; HCO_3^-, 25.0; SO_4^{2-}, 1.99 and dextrose 11.1 (13). Dose-response curves to histamine were made in the presence of cimetidine (1μM) to inhibit concomitant vasodilation via H_2 receptor stimulation. Histamine (0.1mM) caused a maximal increase in vessel tone; all subsequent changes in tone are calculated as a percentage of the response of individual vessel segments to histamine (0.1mM). In experiments wherein tissues were pretreated with PMA, only one dose-response curve to Ca^{2+} was made per segment.

Phorbol 12-Myristate 13-Acetate (PMA) Treatment: Dose response curves to Ca^{2+} (0.1-1.6mM) were made in tissues incubated in Ca^{2+}-free PSS containing 5mM K^+. Responses to Ca^{2+} were compared under control conditions (no PMA) or after pretreatment of basilar arteries for 15 min with PMA (0.1μM). The responses to Ca^{2+} were studied in PMA pretreated tissue segments which were stretched to resting tensions of 300, 500 or 800 mg.

The effect of Bay K 8644 (1.0nM) on dose-response curves to Ca^{2+} in PMA (0.1μM) treated segments was studied by addition of Bay K 8644 to Ca^{2+}-free PSS 10 min prior to readmission of Ca^{2+} (0.1-1.6mM).

Nifedipine Treatment: Dose-response curves to Ca^{2+} (0.1-1.6mM) were made by readmission of Ca^{2+} to tissues pretreated with PMA (0.1μM) and nifedipine (10nM) for 30 min. In some experiments where tissues were pretreated with PMA (0.1μM) alone or with nifedipine (10nM) included, Bay K 8644 (1.0nM) was added for 15 min prior to making dose-response curves to Ca^{2+}.

Staurosporine Treatment: Segments of basilar artery incubated in Ca^{2+}-free PSS were treated with staurosporine (50nM) for 40 min before addition of PMA (0.1μM) for 15 min. Staurosporine (50nM) inhibits PMA-induced augmentation of Ca^{2+}-dependent, stretch-induced tone (14). In some experiments, Bay K 8644 (1.0nM) was also added for 15 min. Dose-response curves to Ca^{2+} (0.1-1.6mM) were made subsequent to these treatments.

RESULTS: Effect of PMA on Basilar Artery Responses to Stretch-Dependent Ca^{2+}-Entry. Histamine (0.1mM) produced a maximal response of 2332 ± 64mg. In tissues pretreated with the inactive phorbol ester 4α-PMA (0.1 and 1.0μM) or the Ca^{2+}-channel agonist Bay K 8644 (1nM and 1μM), readmission of Ca^{2+} (0.1-1.6mM) did not elicit contraction (Fig. 1). Contractile responses to

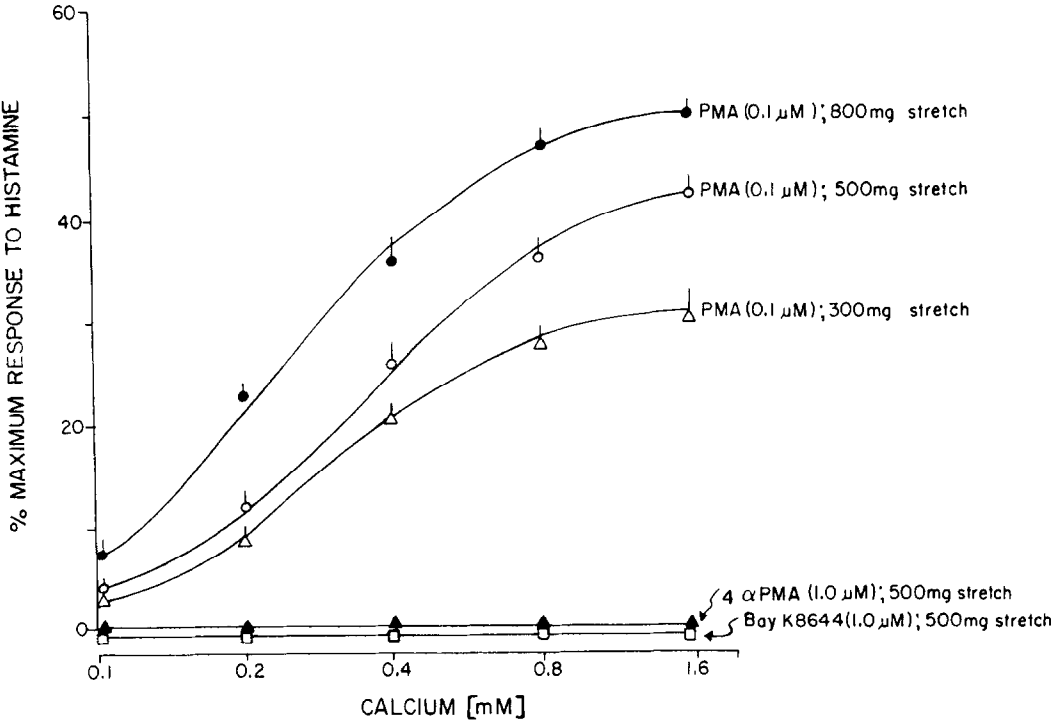

Figure 1: Contractile responses to Ca^{2+} were made by readmitting Ca^{2+} to PMA (0.1μM) treated basilar arteries incubated in Ca^{2+}-free PSS. Artery segments were stretched to either 300mg, 500mg or 800mg; in some segments 4α PMA (1.0μM) or Bay K 8644 (1.0μM) was added to vessels stretched by 500mg tension. Results are expressed as a percent of the tissue maximum response to histamine (0.1mM), which was determined in Ca^{2+}-containing PSS earlier in the experiment.

Ca^{2+} (0.1-1.6mM) were obtained only in PMA (0.1μM) treated segments. The ED_{50} for Ca^{2+} was 0.24 ± 0.04mM. The maximal response to Ca^{2+} (1.6mM) was 631 ± 80mg in tissues stretched by 300mg, 986 ± 48mg in tissues stretched to 500mg and 1113 ± 70mg in tissues stretched to 800mg preload. These contractile responses to Ca^{2+} (1.6mM) represented 31 ± 4, 42 ± 2 and 50 ± 2 percent of the maximal tissue response to histamine, respectively (Fig. 1).

Effect of Bay K 8644 and Staurosporine on Basilar Artery Responses to Ca^{2+} in PMA Treated Tissues. The threshold concentration for vasoconstriction by Bay K 8644 was 5nM. This caused an increase in tension of 90 ± 29mg which was 4 ± 1 percent of the maximal response to histamine. The maximal response to Bay K 8644 (1μM) was 1790 ± 154mg. Fig. 2 shows dose-response curves to Ca^{2+} in tissues pretreated with PMA (0.1μM) alone or in combination with Bay K 8644 (1.0nM). The ED_{50} for Ca^{2+} in tissues pretreated with PMA was 0.27 ± 0.01mM whilst that after addition of Bay K 8644 was 0.12 ± 0.05mM. In tissues pretreated with PMA, the threshold response to Ca^{2+} (0.1mM) was 238 ± 50mg, and that to a maximally effective concentration of Ca^{2+} (1.6mM) was 1209 ± 90mg. The corresponding values in tissues pretreated with PMA and Bay K 8644

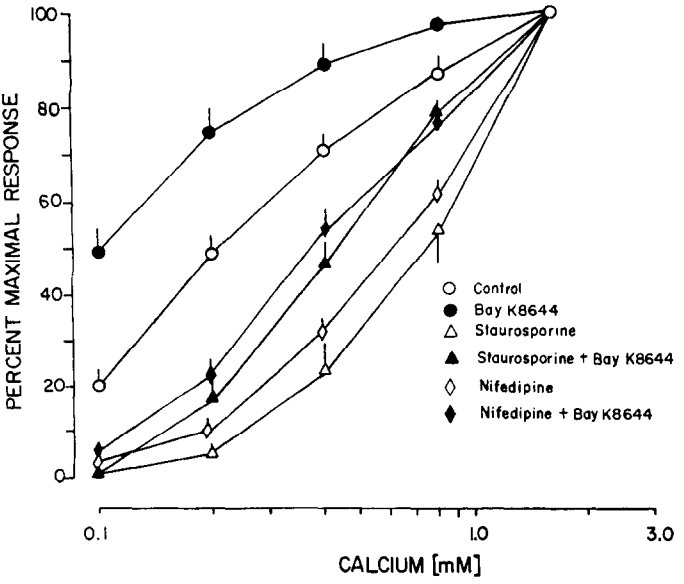

Figure 2: Effect of increases in extracellular Ca^{2+} on tone of rabbit basilar arteries pretreated with PMA (0.1μM; plotted as control curve). In other artery segments also pretreated with PMA (0.1μM), contractile responses to Ca^{2+} were determined in the presence of either Bay K 8644 (1.0nM), staurosporine (50nM), staurosporine (50nM) and Bay K 8644 (1.0nM), nifedipine (10nM) or nifedipine (10nM) and Bay K 8644 (1.0nM). Results are expressed as a percent of the tissue maximum response to histamine (0.1mM) which was determined in Ca^{2+}-containing PSS earlier in the experiment.

were 636 ± 141mg (for 0.1mM Ca^{2+}) and 1348 ± 135mg (for 1.6mM Ca^{2+}). Pretreatment with staurosporine (50nM) reduced the threshold response to Ca^{2+} (0.1mM) in PMA-treated arteries to 8 ± 4mg or 1 ± 0.4 percent of maximal response to histamine. The maximal response to Ca^{2+} (1.6mM) was also reduced to 907 ± 93mg or 55 ± 7 percent of maximal response to histamine. In the presence of Bay K 8644, the response to Ca^{2+} (0.1mM) in PMA and staurosporine treated tissues was 17 ± 2mg or 1 ± 0.8 percent of the maximal response to histamine and to Ca^{2+} (1.6mM) was 1286 ± 158mg or 79 ± 6 percent of the maximal response to histamine.

Effect of Nifedipine on Contractile Response of Basilar Artery to Ca^{2+}. Nifedipine (10nM) caused a significant reduction of contractility to Ca^{2+} in PMA treated arteries (Fig. 2). The maximal response to Ca^{2+} (1.6mM) was 708 ± 102mg and the ED_{50} for Ca^{2+} was 0.58 ± 0.03nM. Addition of Bay K 8644 (1nM) to tissues pretreated with nifedipine and PMA reduced the ED_{50} for Ca^{2+} to 0.39 ± 0.06mM.

DISCUSSION: In this study we demonstrate that contractile responses of the rabbit basilar artery to Ca^{2+} i) occur only after pretreated with PMA, an activator of protein kinase C; ii) do not occur in segments pretreated either with a high concentration of Bay K 8644, a Ca^{2+} channel agonist or 4α-PMA, an inactive analog of PMA; iii) are related to the extent of stretch or preload applied; iv) are inhibited by pretreatment with staurosporine or nimodipine; v) are augmented by low concentrations of Bay K 8644 in tissues pretreated either with staurosporine, nimodipine, or PMA. These results support the hypothesis that protein kinase C increases the intracellular sensitivity of contraction to Ca^{2+} entering via stretch-activated pathways (13,14).

A selective augmentation by protein kinase C activation of stretch-induced, Ca^{2+}-dependent tone in the rabbit facial vein has recently been reported (13). Concentrations of Ca^{2+} insufficient to support tone in untreated stretched segments of rabbit facial vein were maximally effective after PMA-pretreatment (13). In addition, staurosporine, an inhibitor of protein kinase C, produced concentration dependent loss of stretch-induced myogenic tone in this vessel (14). It was proposed that stretch of this myogenically active vessel activated stretch-dependent Ca^{2+}-entry pathways and that protein kinase C interacted with these pathways to increase Ca^{2+} sensitivity or availability. The present study is consistent with the idea that the intracellular sensitivity of the contractile mechanism to Ca^{2+} entering via stretch-activated pathways of the rabbit basilar artery was also increased by protein kinase C activation. The extent of change produced by readmission of Ca^{2+} to the PSS was related to the magnitude of stretch applied

to the vessel. Upon activation of protein kinase C, e.g. by diacylglycerol or PMA, the requirement of Ca^{2+} for cellular activation is reduced.

Contractile responses to Ca^{2+} occur only after activation of protein kinase C by PMA. The extent and sensitivity of tone due to entry of extracellular Ca^{2+} are enhanced by a concentration of the Ca^{2+}-channel agonist Bay K 8644 which by itself does not cause contraction. These observations are partly in accordance with the role of protein kinase C in vascular contraction proposed by Rasmussen, whereby large increases in tone can be supported by previously ineffective concentrations of intracellular Ca^{2+} (7,8). It is likely that the locus of the increased intracellular sensitivity to Ca^{2+} is related to phosphorylation by protein kinase C since a) similar results were not obtained with pretreatment with 4α-TPA and b) these effects were inhibited by staurosporine, an inhibitor of protein kinase C. Responses due to Ca^{2+} in PMA-treated tissues were also attenuated by nifedipine, an inhibitor of Ca^{2+}-entry. The inhibition of Ca^{2+}-dependent tone by nifedipine was reversed by addition of a subthreshold concentration Bay K 8644, supporting the thesis that minor changes in Ca^{2+}-availability produce large increases in tone in PMA-treated vascular smooth muscle.

In conclusion, PMA increases the sensitivity of rabbit basilar artery to extracellular Ca^{2+} induced-tone initiated by stretch. PMA has been demonstrated to increase myofilament sensitivity to Ca^{2+} in chemically skinned coronary arteries and in α-toxin skinned mesenteric artery preparations (12). Our study demonstrates increased vascular sensitivity to Ca^{2+} in intact, unskinned prepartions. The ability of PMA to sensitize intracellular components to Ca^{2+} was related to the magnitude of stretch applied, and was sensitive to staurosporine, an inhibitor of protein kinase C (15). The ability of staurosporine to attenuate Ca^{2+}-dependent tone was overcome by promoting influx of extracellular Ca^{2+} with Bay K 8644. In addition to implicating an increase in Ca^{2+}-dependent protein kinase C activity during maintained stretch-induced vascular tone, our results confirm the role of extracellular Ca^{2+} entry via plasma membrane stretch-dependent Ca^{2+}-channels during maintained vascular tone in this cerebral artery.

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