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STAUROSPORINE, A PROTEIN KINASE C INHIBITOR, ATTENUATES Ca^{2+}-DEPENDENT STRETCH-INDUCED VASCULAR TONE*

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The effects of protein kinase C inhibition by staurosporine was studied on Ca-dependent tone of the rabbit facial vein. Tone was produced either by stretch or by readmission of Ca^{2+} in a non-depolarizing Ca^{2+}-free salt solution. Stretch-induced tone was inhibited by staurosporine. When tissues were incubated in a Ca^{2+}-free solution, staurosporine (50nM) inhibited the contractile responses produced by readmission of Ca^{2+}. These observations suggest that maintenance of stretch-induced extracellular Ca^{2+}-dependent tone may be regulated by protein kinase C.

The response of vascular smooth muscle to vasoconstrictors is biphasic: an initial rapid contraction thought to be due to intracellular release of Ca^{2+} is followed by a slowly developing extracellular Ca^{2+}-dependent tonic contraction (1). These two components have been proposed to be regulated by products of phosphatidylinositol - 4,5 bisphosphate hydrolysis whereby myo-inositol 1,4,5-triphosphate causes the release of Ca^{2+} from intracellular stores and diacylglycerol leads to activation of protein kinase C, which through a series of phosphorylations leads to maintenance of vascular contraction (2).

The effect of activation of protein kinase C in vitro, e.g. by PMA, and its inhibition, e.g. by compounds such as H7, on vascular contraction is usually studied in large arteries using receptor agonists or membrane

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

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depolarization by K^+ (3). Such vessels lack intrinsic or myogenic tone, which is a primary determinant of vascular resistance in vivo (4). It is clear that the cellular regulation of Ca^{2+}-dependent intrinsic tone is distinct from Ca^{2+}-dependent tone, due for example, to norepinephrine, histamine and K^+ since a) the effects of Ca^{2+}-entry inhibitors on the two modes of tone are not the same (5) and b) protein kinase C activation by PMA augments stretch-dependent tone at concentrations which have a depressant effect on tone due to K^+ or histamine (6).

In this study we report on the effect of staurosporine, an inhibitor of protein kinase C (7) on stretch-induced tone of the rabbit facial vein. This vessel segment is involved in cranial thermoregulation, and exhibits features that can be used to advantage since only the stretch-induced tone component is temperature dependent (8).

METHODS

Rings of facial veins were dissected from male adult rabbits (4.5 - 5.5 Kg) and prepared for in vitro isometric force measurements (6,8). Experiments were made in a physiological salt solution (PSS) of the following composition (in mM): Na^+, 144.2; K^+, 4.9; Ca^{2+}, 1.6; Mg^{2+}, 1.2; Cl^-, 126.7; HCO_3^-, 25.0; SO_4^{2-}, 1.19; glucose, 11.1; EDTA, 0.024. The PSS was gassed with 95% O_2/5% CO_2 and had a pH of 7.4. Tissue segments were placed in a double-jacketed 3 ml tissue bath containing PSS maintained at a constant temperature. Staurosporine was purchased from Kyowa Hakko U.S.A. Inc. (New York, NY) and PMA from Sigma (St. Louis, MO) and stock solutions were prepared in dimethylsulfoxide.

Stretch-induced tone

Vessel segments were stretched to 0.5g and equilibrated for 30 min in PSS maintained at 32°C. After a maintained response to this stretch was established, papaverine (10μM) was added for 3 mins to determine the extent of stretch-induced tone. The PSS was then replaced several times and then the thermostat on the heater-circulator pump was reset to 42°C. The subsequent changes in temperature and vascular tone were noted on the strip chart recorder. When increases in vascular tone were maximal, usually at 40°C, papaverine (10μM) was added for 3 mins and the loss of tone, representing that due to stretch, was measured. After papaverine was washed out and maximal tone regained, the effects of staurosporine on stretch-induced tone was studied at 40°C by making cumulative additions of staurosporine (1nM to 0.1mM) and recording changes in magnitude of stretch-induced tone.

The effect of staurosporine on stretch-induced, papaverine-sensitive tone was studied at 40°C using two protocols. After the maximal dilator response

to papaverine (10μM) had been quantitated, tissues were unstretched and treated for 30 minutes with staurosporine (50nM). Thereafter a 0.5g stretch was applied and the ensuing papaverine (10μM) induced loss of tone expressed as a percentage of the control response. In other experiments tissues were equilibrated in Ca^{2+}-free PSS and dose-response curves made to readmission of Ca^{2+} (0.1 to 1.6mM) before and after a 30 min incubation of staurosporine (50nM). Time dependent changes in tissue sensitivity were determined by carrying out parallel experiments when dimethylsulfoxide, the solvent vehicle for staurosporine was added alone.

RESULTS

Effect of staurosporine on stretch-induced tone

Papaverine (10μM) produced a loss of tone in vessels stretched in PSS at 40°C, and not in vessels stretched at 32°C. When cumulative additions of staurosporine were made, loss of tone occurred only in tissues with a stretch-induced, papaverine-sensitive tone (Fig. 1). The IC_{50} for staurosporine was 8.6 (±0.18) x 10^{-7}M (n=10). Activation of protein kinase C by PMA (0.1μM) caused a contraction in tissues with stretch-induced tone; pretreatment with staurosporine prevented the contraction caused by PMA (Fig. 1).

Pretreatment of rabbit facial vein segments with staurosporine (50nM) reduced the maximal dilator response to papaverine (10μM) to 48.9 ± 7.7 (n=12) percent of the control response. Tissues with stretch-induced tone relaxed when placed in a Ca^{2+}-free PSS. Upon readmission of Ca^{2+}, a dose-dependent

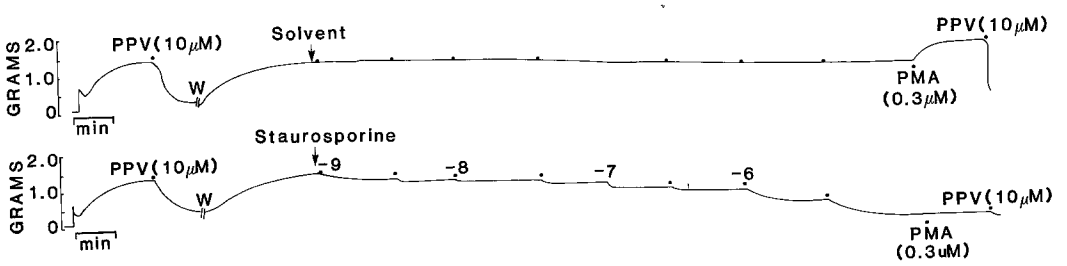

Figure 1: Tracings of experiment wherein two ring segments of rabbit facial vein were stretched (0.7g) in physiological salt solution (42°C). The extent of active, stretch-induced tone is determined from the vasodilator response to papaverine (PPV, 10μM), which was subsequently washed out (W). When active tone was regained, a cumulative dose-response curve to staurosporine was made in one segment (lower trace) while the other segment received appropriate volumes of solvent (DMSO). PMA (0.3μM)-induced tone only in tissues not pretreated with staurosporine.

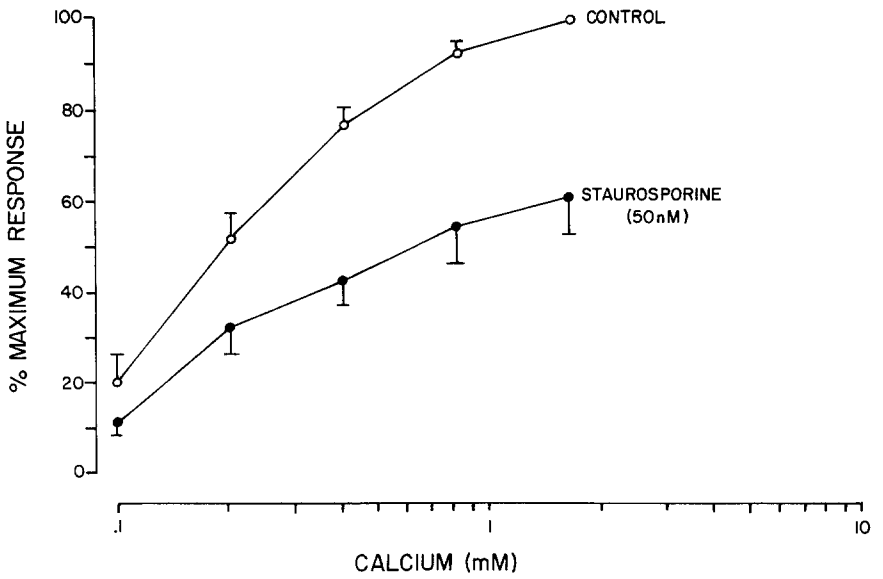

Figure 2: Dose-response curve for readmission of Ca^{2+} in rabbit facial vein segments. Tissue segments were stretched (0.7g) in physiological salt solution (PSS, 42°C) not containing Ca^{2+}. Active tone developed upon readmission of Ca^{2+} (control); tissues were washed in Ca^{2+}-free PSS, pretreated with staurosporine (50nM) and responses to Ca^{2+} determined again.

increase in tone occurred (Fig. 2). The ED_{50} for Ca^{2+} was 0.20 ± 0.06mM (n=20) in untreated segments. Staurosporine (50nM) pretreatment significantly inhibited the magnitude of contraction observed with each concentration of Ca^{2+} (Fig. 2). The ED_{50} for Ca^{2+} in staurosporine (50nM) pretreated tissues was 0.30 ± 0.13mM (n=10). The maximal increase in tone occurring with Ca^{2+} (1.6mM) was 1025 ± 178 mg in control tissues and 613 ± 104 mg in the presence of staurosporine (50nM).

DISCUSSION

In this study we report on the effects of staurosporine, a putative inhibitor of protein kinase C, on stretch-induced tone. Tone due to stretch of the rabbit facial vein was inhibited dose-dependently by staurosporine. When stretch-induced tone was studied by re-admission of Ca^{2+} to the PSS, staurosporine inhibited the ability of Ca^{2+} to increase tone while not decreasing the sensitivity (ED_{50}) to Ca^{2+}. These findings support our previous suggestion that stretch-induced vascular tone involves protein kinase C activation.

A role for protein kinase C in maintenance of vascular tone has been proposed in a number of studies. Such studies primarily utilized activators

of protein kinase C such as PMA and an observation from such experiments is that once activated, protein kinase C is fully able to maintain tonic vascular contraction, e.g. to receptor agonists (2). In this study we show that staurosporine is able to decrease stretch-induced vascular tone. This effect of staurosporine is manifest when added to tissues with stretch-induced tone (Fig. 1) or when added prior to readmission of Ca^{2+} to stretch-segments of tissue (Fig. 2), in which case Ca^{2+}-entry has been proposed to occur as a result of stretch-mediated mechanisms (9). It is unlikely that staurosporine is acting as a Ca^{2+}-entry inhibitor, since organic inhibitors of Ca^{2+}-entry such as verapamil, diltiazem and nifedipine paradoxically augment stretch-induced vascular tone in the rabbit facial vein (9). In addition, it has recently been shown that H7, an inhibitor of protein kinase C, inhibits K^{+}-induced tone without altering $^{45}Ca^{2+}$-influx (3). It is also unlikely that staurosporine inhibits stretch-induced tone by inhibiting cyclic AMP dependent protein kinase, since in the rabbit facial vein increases in cyclic AMP, e.g. by caffeine, theophylline and forskolin result in vasodilation (Laher and Bevan, unpublished observations). The results of this study suggest that Ca^{2+}-dependent stretch-induced tone in the rabbit facial vein occurs via a pathway that is sensitive to staurosporine, a potent inhibitor of protein kinase C (7,10).

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