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European Journal of Pharmacology 340 (1997) 203–208

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# Intracellular $Ca^{2+}$ elevation and contraction due to prostaglandin $F_{2\alpha}$ in rat aorta

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Received 6 October 1997; accepted 10 October 1997

**Abstract**

Prostaglandin $F_{2\alpha}$ was tested to determine (a) whether its effect on intracellular $Ca^{2+}$ levels ($[Ca^{2+}]_i$) and force in vascular smooth muscle was mediated through activation of the thromboxane $A_2$ and/or prostaglandin receptor, and (b) the relative roles of $Ca^{2+}$ influx via L-type and non-L-type $Ca^{2+}$ channels in prostaglandin receptor-mediated contraction. $[Ca^{2+}]_i$ and force were measured simultaneously in fura-2-loaded rat aortic strips. The thromboxane $A_2$ receptor antagonist, SQ29548 ([1*S*]-1a,2b(5*Z*),3b,4a-7-(3-{2-[(phenylamino)carbonyl] hydrazinomethyl)-7-oxobicyclo-[2.2.1]hept-2-yl-5-heptenoic acid), prevented the prostaglandin $F_{2\alpha}$-induced plateau $[Ca^{2+}]_i$ elevation and force by 80–90%, while abolishing these responses due to the thromboxane $A_2$ receptor agonist, U46619 (9,11-dideoxy-9$\alpha$,11$\alpha$-methanoepoxy prostaglandin $F_{2\alpha}$). Prostaglandin $F_{2\alpha}$ (+SQ29548)-induced plateau $[Ca^{2+}]_i$ elevation and force were not inhibited by verapamil. $Ni^{2+}$, a non-selective cation channel blocker, in the presence of verapamil, abolished the prostaglandin $F_{2\alpha}$ (+SQ29548)-elevated $[Ca^{2+}]_i$, while the contraction was only partially inhibited. These results suggest that, in rat aorta, (1) elevated $[Ca^{2+}]_i$ and force due to high prostaglandin $F_{2\alpha}$ concentrations largely results from thromboxane $A_2$ receptor activation, and (2) the prostaglandin component of the prostaglandin $F_{2\alpha}$-induced contraction is dependent on $Ca^{2+}$ influx via non-L-type channels. © 1997 Elsevier Science B.V.

*Keywords:* U46619; SQ29548; Fura-2; Smooth muscle, vascular

## 1. Introduction

The role of intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) in prostaglandin receptor-mediated contraction of vascular smooth muscle is not clear. Several investigators demonstrated that prostaglandin $F_{2\alpha}$ elevated $[Ca^{2+}]_i$ levels in vascular tissue (Sato et al., 1988; Hisayama et al., 1990; Ozaki et al., 1990; Balwierczak, 1991; Hori et al., 1992; Hori et al., 1993; Makujina et al., 1995). However, binding and contractility studies indicate that prostaglandin $F_{2\alpha}$ concentrations greater than 100 nM activate not only prostaglandin receptors, but also the thromboxane $A_2$ receptor (Hanasaki and Arita, 1989; Kato et al., 1990; Dorn et al., 1992; Rapoport, 1993). Thus, while prostaglandin $F_{2\alpha}$ concentrations $\geq 2$ $\mu$M, the lowest concentration used in all (Sato et al., 1988; Hisayama et al., 1990; Ozaki et al., 1990; Balwierczak, 1991; Hori et al., 1992; Hori et al., 1993) but one report (Makujina et al., 1995), elevated $[Ca^{2+}]_i$ and induced contraction in ferret and rat aorta, and pig coronary artery, these effects likely resulted from both prostaglandin and thromboxane $A_2$ receptor activation. Furthermore, in the only report that tested the effect of a low prostaglandin $F_{2\alpha}$ concentration (10 nM), little increase in $[Ca^{2+}]_i$ and force was observed, as determined in rat aorta (Makujina et al., 1995).

Another possible reason for the lack of clarity regarding the role of $[Ca^{2+}]_i$ in prostaglandin receptor-mediated contraction of vascular smooth muscle, stems from the only investigation that reported the effects of prostaglandin $F_{2\alpha}$ on prostaglandin receptor-mediated $[Ca^{2+}]_i$ elevation in the presence of thromboxane $A_2$ receptor blockade

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(Dorn et al., 1992). These authors demonstrated that the selective thromboxane $A_2$ receptor antagonist, SQ29548, did not alter prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ elevation in cultured rat aorta smooth muscle cells. Thus, since SQ29548 nearly abolished prostaglandin $F_{2\alpha}$-induced contraction in intact rat aorta (Dorn et al., 1992; Rapoport, 1993), these results would suggest that $[Ca^{2+}]_i$ elevated in response to prostaglandin $F_{2\alpha}$ may not be related to contraction.

It should be considered, however, that our interpretation of an apparent dissociation between prostaglandin receptor-mediated $[Ca^{2+}]_i$ elevation and contraction may have resulted from the measurement of $[Ca^{2+}]_i$ and force in different preparations: cultured rat aorta smooth muscle cells and intact rat aorta, respectively (Dorn et al., 1992). Thus, considering (1) the lack of knowledge concerning the contributions of thromboxane $A_2$ and prostaglandin receptor-activation to prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ elevation and contraction, and (2) the possibility that prostaglandin receptor-mediated $[Ca^{2+}]_i$ elevation was entirely unrelated to contraction, we investigated the relative contribution of prostaglandin and thromboxane $A_2$ receptor activation to prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ elevation and contraction in intact rat aorta. In addition, since there are no studies that directly investigate the source of $Ca^{2+}$ that may contribute to prostaglandin receptor-mediated contraction, we investigated whether $Ca^{2+}$ influx via L-type and non-L-type $Ca^{2+}$ channels may be responsible for the contraction.

## 2. Materials and methods

Rats (Sprague–Dawley, male, 250–350 g) were asphyxiated with $CO_2$ and the thoracic aorta removed and cleaned of extraneous fatty tissue. Each aorta was cut into helical strips ($2 \times 10$ mm), the endothelium removed, and the strip mounted vertically on a holder attached to an isometric force transducer. Preliminary results demonstrated that U46619-induced contraction, as well as $EC_{50}$ values, were similar in strips and ring segments normalized to cross-sectional area. The holder containing the strip was then placed in a cuvette containing Krebs–Ringer bicarbonate solution (Rapoport, 1987) plus 0.2 mM neostigmine, 1 mM probenecid, 0.02% pluronic F-127, and 5 $\mu$M fura-2/AM. Tissue was placed under 20 mN resting tension and was

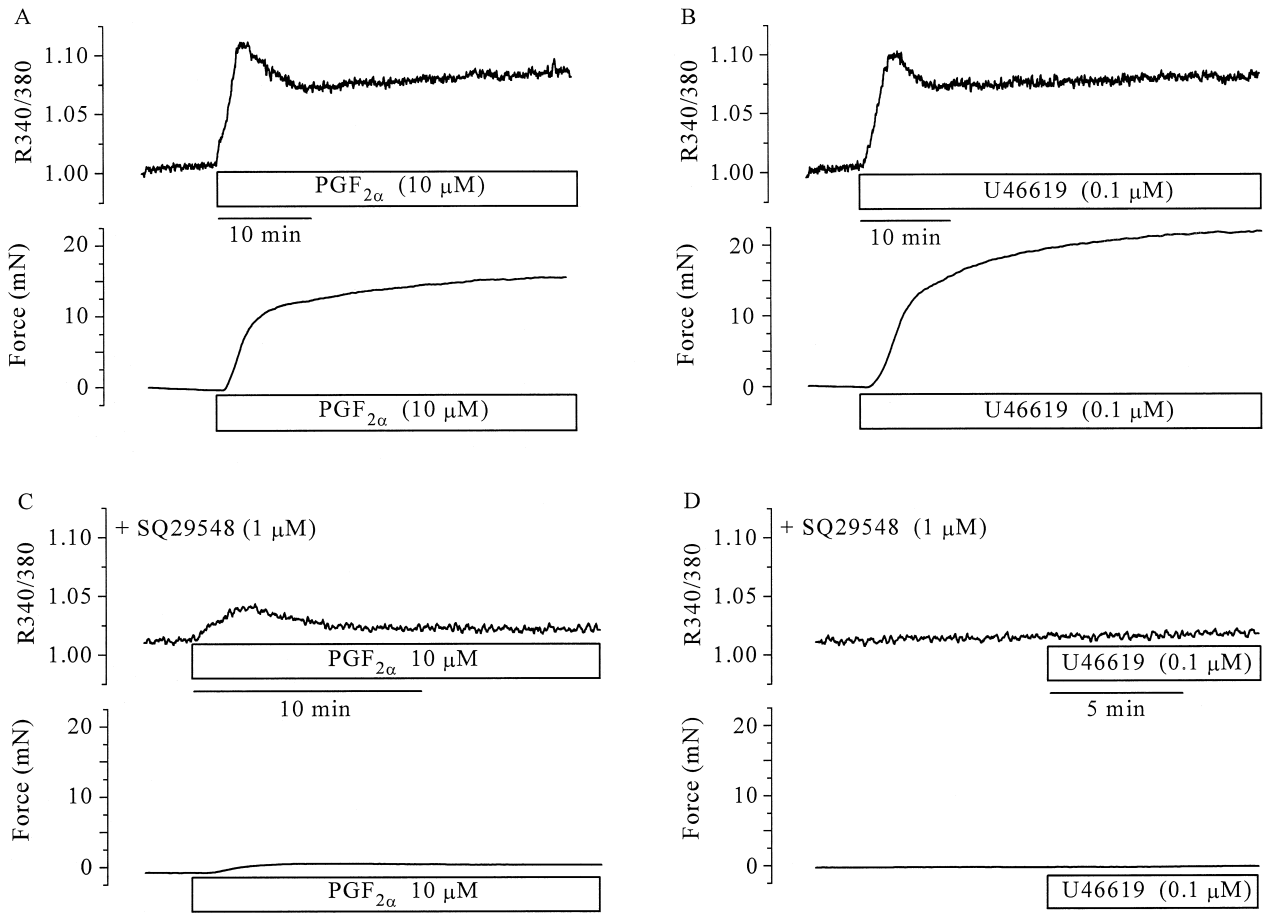


Fig. 1. Effects of prostaglandin $F_{2\alpha}$ and U46619 on $[Ca^{2+}]_i$ and contractile force in the absence and presence of SQ29548 in rat aorta. Typical tracings of simultaneous changes in $[Ca^{2+}]_i$ and force due to 10 $\mu$M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the absence (A) and presence of 1 $\mu$M SQ29548 (C), and to 0.1 $\mu$M U46619 in the absence (B) and presence of 1 $\mu$M SQ29548 (D) are shown. $[Ca^{2+}]_i$ and force are expressed as R340/380 and mN, respectively.

incubated in the dark for 2.5–3 h at room temperature with sonication applied external to the cuvette. The cuvette was then placed in a water-jacketed sample chamber holder (37°C) and resting tension readjusted to 20 mN. The tissue was perfused (12 ml/min) with 37°C gassed Krebs–Ringer bicarbonate solution containing 3 $\mu$M indomethacin and 1 mM probenecid and allowed to equilibrate for 30 min prior to agent addition. The intimal surface of fura-2 loaded tissue was subjected to excitation wavelengths of 340 and 380 nm. Emitted fluorescence was measured at 510 nm using a PTI Deltascan-1 spectrofluorometer configured for front-face fluorescence (Photon Technology International).

Contractile force was reported in mN and measured simultaneously with $[Ca^{2+}]_i$. The ratio of 340–380 nm excitation (R340/380) is reported as a relative measure of free $[Ca^{2+}]_i$. In some tissues, the absolute $[Ca^{2+}]_i$ was calculated assuming an apparent dissociation constant ($K_d$) of the fura-2:$Ca^{2+}$ complex of 224 nM using the formula derived by Grynkiewicz et al. (1985). Maximal and minimal R340/380 were determined by 10 $\mu$M ionomycin addition, followed by $Ca^{2+}$-free solution containing 2 mM EGTA, respectively. $MnCl_2$ (5 mM) was added at the end of each experiment to determine autofluorescence which was subtracted from the experimental values. It should be noted that it is difficult to determine absolute $[Ca^{2+}]_i$ due to changes in the $K_d$ of the fura-2:$Ca^{2+}$ complex in the cytoplasm, as well as incomplete effects of ionomycin.

### 2.1. Statistical methods

Differences between means were analyzed using Student's unpaired *t*-test. Significance was accepted at the 0.05 level of probability. Shown are means ± S.E.M. *n* represents the number of animals.

### 2.2. Drugs

Reagent sources were as follows: Sigma: indomethacin, ionomycin, neostigmine methyl sulfate, probenecid, nickel chloride; Biomol: verapamil; Cayman: prostaglandin $F_{2\alpha}$ tromethamine, [1*S*]-1a,2b(5*Z*),3b,4a-7-(3-{2-[(phenylamino)carbonyl] hydrazinomethyl)-7-oxobicyclo-[2.2.1]hept-2-yl-5-heptenoic acid (SQ29548); Molecular Probes: fura-2 acetoxymethyl (AM) ester, pluronic F-127; Upjohn (gift): 9,11-dideoxy-9$\alpha$,11$\alpha$-methanoepoxy prostaglandin $F_{2\alpha}$ (U46619).

## 3. Results

### 3.1. Effects of 10 $\mu$M prostaglandin $F_{2\alpha}$ and 0.1 $\mu$M U46619 on simultaneous changes in contractile force and $[Ca^{2+}]_i$ elevation

Prostaglandin $F_{2\alpha}$ (10 $\mu$M) and U46619 (0.1 $\mu$M; 9,11-dideoxy-9$\alpha$,11$\alpha$-methanoepoxy prostaglandin $F_{2\alpha}$)

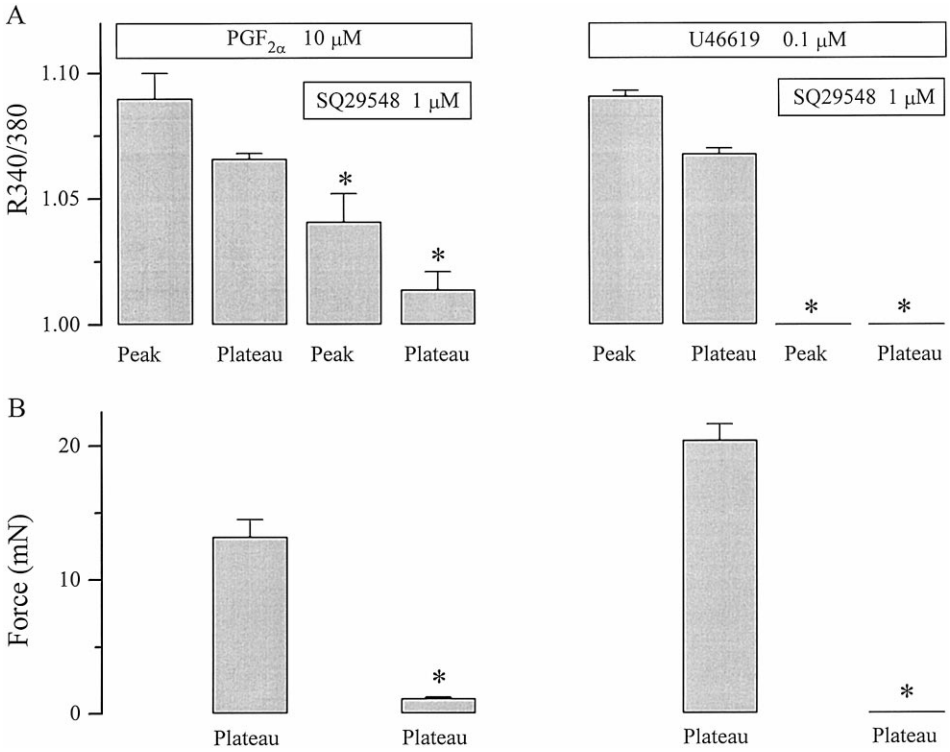


Fig. 2. Effects of SQ29548 on prostaglandin $F_{2\alpha}$- and U46619-induced $[Ca^{2+}]_i$ elevation and contraction in rat aorta. Peak and plateau $[Ca^{2+}]_i$ (A) and force (B) in response to 10 $\mu$M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (left panels) and 0.1 $\mu$M U46619 (right panels) in the presence and absence of 1 $\mu$M SQ29548 are shown. $[Ca^{2+}]_i$ and force are expressed as R340/380 and mN, respectively. Shown are means ± S.E.M. ($n = 3$ in each case). * Significantly less than corresponding value in the absence of SQ29548.

induced rapid increases in force followed by a plateau phase (Fig. 1). The magnitude of force of the plateau phase due to 10 $\mu$M prostaglandin $F_{2\alpha}$ tended to be less than that due to 0.1 $\mu$M U46619, although this difference was not statistically significant (Figs. 1 and 2).

Prostaglandin $F_{2\alpha}$ (10 $\mu$M) and 0.1 $\mu$M U46619 induced a rapid, somewhat variable transient increase in $[Ca^{2+}]_i$, which was followed by a plateau response (Fig. 1). The magnitude of plateau $[Ca^{2+}]_i$ elevation to 10 $\mu$M prostaglandin $F_{2\alpha}$ was similar to that of 0.1 $\mu$M U46619 (Figs. 1 and 2). Prostaglandin $F_{2\alpha}$ (10 $\mu$M)- and 0.1 $\mu$M U46619-elevated $[Ca^{2+}]_i$ levels were 205 nM (mean of 190 and 220 nM) and 217 nM (mean of 188 and 246 nM), respectively, over basal, which was 52 ± 3 nM (mean ± S.E.M.; $n = 10$).

### 3.2. Effects of thromboxane $A_2$ receptor antagonism with SQ29548

SQ29548 (1 $\mu$M; [1*S*]-1a,2b(5*Z*),3b,4a-7-(3-{2-[(phenylamino)carbonyl] hydrazinomethyl)-7-oxobicyclo-

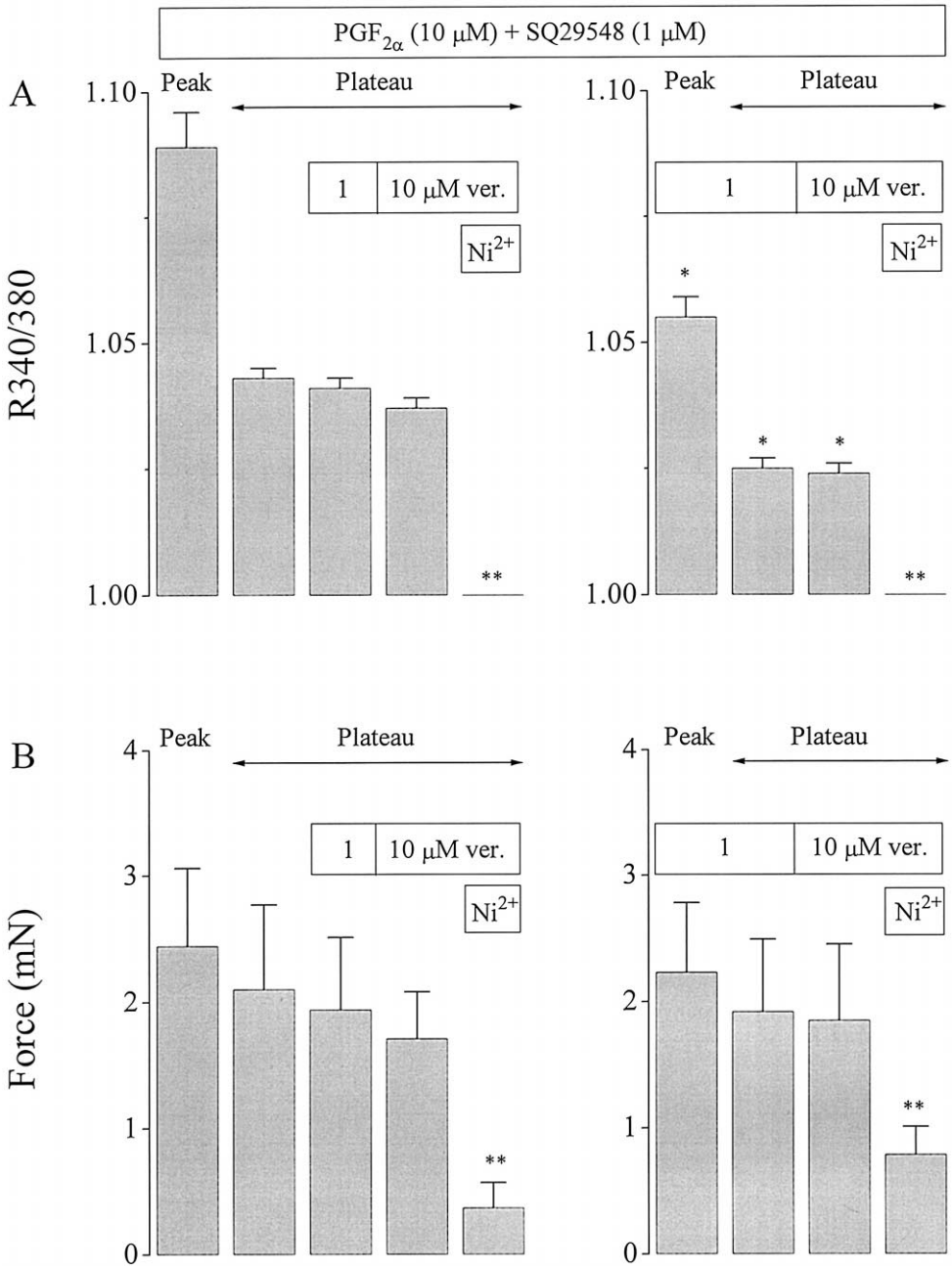


Fig. 3. Effects of verapamil and $Ni^{2+}$ on prostaglandin receptor-mediated $[Ca^{2+}]_i$ elevation and contraction in rat aorta. Peak and plateau $[Ca^{2+}]_i$ (A) and force (B) in response to 10 $\mu$M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the presence of 1 $\mu$M SQ29548 are shown. Aorta remained untreated (left panels) or was pretreated with 1 $\mu$M verapamil (right panels). Verapamil (ver.) and 1 mM $Ni^{2+}$ in the continued presence of verapamil was added during the plateau response as indicated. $[Ca^{2+}]_i$ and force are expressed as R340/380 and mN, respectively. Shown are means ± S.E.M. ($n = 4$) in each case. * Significantly less than corresponding value in the absence of verapamil pretreatment. ** Significantly less than in the absence of $Ni^{2+}$.

[2.2.1]hept-2-yl-5-heptenoic acid) greatly prevented the 10 $\mu$M prostaglandin $F_{2\alpha}$-induced increase in force (Figs. 1 and 2; see also Rapoport, 1993). In contrast to the partial inhibitory effect of SQ29548 on the prostaglandin $F_{2\alpha}$-induced contraction, SQ29548 completely prevented contraction due to 0.1 $\mu$M U46619 (Figs. 1 and 2; see also Rapoport, 1993).

SQ29548 greatly, but not completely, prevented the transient and plateau $[Ca^{2+}]_i$ elevations in response to 10 $\mu$M prostaglandin $F_{2\alpha}$ (Figs. 1 and 2). Similar results were observed with a lower SQ29548 concentration (0.3 $\mu$M; data not shown). Prostaglandin $F_{2\alpha}$ (10 $\mu$M) elevated $[Ca^{2+}]_i$ in the presence of 1 $\mu$M SQ29548 was $24 \pm 3$ and $16 \pm 2$ nM for peak and plateau responses, respectively (mean $\pm$ S.E.M.; $n = 4$), over basal. In contrast, SQ29548 completely prevented the $[Ca^{2+}]_i$ elevation due to 0.1 $\mu$M U46619 (Figs. 1 and 2).

### *3.3. Effects of verapamil and $Ni^{2+}$ in the presence of SQ29548*

Verapamil did not inhibit $[Ca^{2+}]_i$ elevation and contraction when added during the prostaglandin receptor-mediated plateau response (Fig. 3). $Ni^{2+}$ (1 mM) in the continued presence of verapamil, abolished the plateau $[Ca^{2+}]_i$ elevation, while some contraction remained (Fig. 3).

Verapamil pretreatment inhibited prostaglandin receptor-mediated peak and plateau $[Ca^{2+}]_i$ elevation by $\sim$ 40%, while contraction remained unaltered (Fig. 3). $Ni^{2+}$ (1 mM) abolished the $[Ca^{2+}]_i$ elevation and partially inhibited contraction in vessels pretreated with verapamil (Fig. 3).

## 4. Discussion

The present study demonstrates that maximal prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ and contraction in rat aorta are due, almost entirely, to thromboxane $A_2$ receptor activation. This conclusion is supported by the observations that the selective thromboxane $A_2$ receptor antagonist, SQ29548 (Dorn et al., 1992), decreased both the steady state $[Ca^{2+}]_i$ and contraction in response to 10 $\mu$M prostaglandin $F_{2\alpha}$ by 80–90%. The magnitude of force due to 10 $\mu$M prostaglandin $F_{2\alpha}$ in the presence of SQ29548 was similar to the magnitude of force due to 100 nM prostaglandin $F_{2\alpha}$ in the absence of SQ29548 (Fig. 2 compared with Fig. 1 of Rapoport, 1993), suggesting that 100 nM prostaglandin $F_{2\alpha}$ maximally activates prostaglandin receptor.

With respect to the relative contribution of prostaglandin and thromboxane $A_2$ receptor activation to the prostaglandin $F_{2\alpha}$-elevated $[Ca^{2+}]_i$, in intact rat aorta the majority (80–90%) of maximal prostaglandin $F_{2\alpha}$ $[Ca^{2+}]_i$ elevation can be attributed to thromboxane $A_2$ receptor activation (present results). In contrast, in cultured rat aorta smooth muscle cells, thromboxane $A_2$ receptor activation apparently does not contribute to prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ elevation since thromboxane $A_2$ receptor blockade by SQ29548 did not decrease prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ elevation (Dorn et al., 1992). Thus, our interpretation of an apparent dissociation between prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ and contraction, based on the findings that SQ29548 inhibited prostaglandin $F_{2\alpha}$-induced contraction of intact aorta (Dorn et al., 1992; Rapoport, 1993) and yet did not inhibit prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ elevation in cultured aorta smooth muscle cells (Dorn et al., 1992), may be explained by the apparent lack of contribution of thromboxane $A_2$ receptor activation to prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ elevation in cultured cells.

The above contrast between intact aorta and cultured aorta smooth muscle cells with respect to the relative contribution of prostaglandin and thromboxane $A_2$ receptor activation to prostaglandin $F_{2\alpha}$-induced $[Ca^{2+}]_i$ elevation is likely due to both decreased thromboxane $A_2$ receptor-mediated $[Ca^{2+}]_i$ elevation, and increased prostaglandin receptor-mediated $[Ca^{2+}]_i$ elevation, in cultured cells as compared to intact tissue. This conclusion follows from the observations that (1) maximal prostaglandin receptor-mediated $[Ca^{2+}]_i$ elevation due to prostaglandin $F_{2\alpha}$ was 200 nM in cultured cells (Dorn et al., 1992), and 24 nM in intact aorta (present results), and (2) maximal thromboxane $A_2$ receptor-mediated $[Ca^{2+}]_i$ elevation due to the thromboxane $A_2$ receptor agonist, U46619, was 25 nM in cultured cells (Dorn et al., 1992), and was 217 nM in response to U46619 and prostaglandin $F_{2\alpha}$ in intact aorta (present results).

With regard to the source of $Ca^{2+}$ influx associated with prostaglandin receptor-mediated contraction, the influx does not occur via L-type $Ca^{2+}$ channels. This conclusion is supported by the observations that treatment with a verapamil concentration as high as 10 $\mu$M either prior to or during the plateau contraction was without effect. Verapamil (10 $\mu$M) completely inhibited 33.2 mM KCl-induced contraction and $[Ca^{2+}]_i$ elevation (manuscript submitted). In contrast, $Ni^{2+}$, which is a non-selective cation channel blocker, in the presence of verapamil and SQ29548, greatly inhibited the prostaglandin $F_{2\alpha}$-induced contraction and abolished the elevated $[Ca^{2+}]_i$. It is unlikely that $Ni^{2+}$ inhibited the contraction and $[Ca^{2+}]_i$ elevation due to a direct intracellular action since $Ni^{2+}$ does not gain access to the cytosol (Merritt et al., 1989). Thus, these results represent the novel demonstration, i.e., the measurement of both force and $[Ca^{2+}]_i$, of the apparent coupling between agonist-induced $Ca^{2+}$ influx via non-L-type $Ca^{2+}$ channels and force in prostaglandin receptor activation. Consistent with these results, $Ca^{2+}$ influx via non-L-type $Ca^{2+}$ channels in norepinephrine-induced contraction in rat aorta has been suggested based upon the partial inhibition by nisoldipine of the contraction and $^{45}Ca^{2+}$ influx (Morel and Godfraind, 1991). It should be noted that while $Ni^{2+}$ abolished $[Ca^{2+}]_i$ elevation, con-

traction was not completely inhibited. Thus, additional mechanisms independent of $[Ca^{2+}]_i$ elevation may be partly responsible for prostaglandin receptor-mediated contraction.

The explanation is not clear as to why verapamil pretreatment inhibited prostaglandin receptor-mediated plateau (and peak) $[Ca^{2+}]_i$ elevation, while verapamil addition during the plateau response was without effect. Furthermore, $Ca^{2+}$ influx via L-type $Ca^{2+}$ channels was apparently not coupled to prostaglandin receptor-mediated contraction, since the decrease in $[Ca^{2+}]_i$ elevation due to verapamil pretreatment was not associated with decreased contraction.

It should also be noted that one or more prostaglandin receptors may be responsible for the prostaglandin $F_{2\alpha}$-induced contraction elicited in the presence of thromboxane $A_2$ receptor antagonist, since binding studies demonstrated that [$^3$H]prostaglandin $E_2$ and [$^3$H]prostaglandin $F_{2\alpha}$ were displaced by non-labeled prostaglandin $F_{2\alpha}$ with similar affinities in cultured rat aorta smooth muscle cells (Hanasaki and Arita, 1989; Dorn et al., 1992). In addition, the lack of selective antagonists for the prostaglandin $F_{2\alpha}$ receptor (Coleman et al., 1994) makes conclusions tenuous with respect to the prostaglandin receptor responsible for the thromboxane $A_2$ receptor-independent component of the prostaglandin $F_{2\alpha}$-induced contraction and $[Ca^{2+}]_i$ elevation.

In summary, the present results demonstrate that, at least in the rat aorta, prostaglandin $F_{2\alpha}$-induced contraction and $[Ca^{2+}]_i$ are almost entirely mediated through the thromboxane $A_2$ receptor. Prostaglandin $F_{2\alpha}$ also induces contraction and $[Ca^{2+}]_i$ elevation, albeit to a smaller magnitude, via a prostaglandin receptor(s). The prostaglandin receptor-mediated contraction appears related to $Ca^{2+}$ influx via non-L-type $Ca^{2+}$ channels.

## Acknowledgements

This work was supported in part by grants from the Department of Veterans Affairs (R.M.R.), NIH HL23240 (R.J.P.), and a predoctoral fellowship from Ege University (M.T.).

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