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SHEAR STRESS-INDUCED CALCIUM TRANSIENTS IN ENDOTHELIAL CELLS FROM HUMAN UMBILICAL CORD VEINS

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SUMMARY

1. Changes of the free cytosolic Ca^{2+} concentration induced by shear stress were measured in Fura-2 acetoxymethyl ester-loaded endothelial cells from human umbilical cord veins.

2. We were able to induce Ca^{2+} transients in almost every cell by blowing a stream of physiological solution onto a single endothelial cell thereby inducing shear stress between 0 and 50 dyn cm^{-2}. The Ca^{2+} response could be graded by varying the shear stress, and reached a half-maximal value at a shear stress of 30 dyn cm^{-2}.

3. The shear stress responses critically depended on the extracellular Ca^{2+} concentration and were absent in a Ca^{2+}-free solution. Repetitive application of short pulses of shear stress induced cumulative effects because of the slow decay of the shear stress Ca^{2+} responses (time constants $82{\cdot}3 \pm 17{\cdot}8$ s from twenty-five cells). Application of a depolarizing high potassium solution to reduce the driving force for Ca^{2+} entry decreased the Ca^{2+} transients in some of the cells.

4. Application of shear stress in the presence of other divalent cations, such as nickel, cobalt or barium, always produced substantial changes in the ratio of the 390/360 nm fluorescence signal, indicating influx of these cations and subsequent quenching of the Fura-2 fluorescence.

5. Shear stress responses in the presence of 10 mM Ca^{2+} were completely blocked by application of 1 mM La^{3+}.

6. Incubation of the cells with the phorbol ester 12-*O*-tetradeconoyl phorbol-13-acetate (TPA) did not alter the shear stress response, but completely blocked histamine-induced Ca^{2+} transients.

7. Small submaximal shear stress potentiated the Ca^{2+} transients induced by histamine.

8. We conclude that shear stress-dependent Ca^{2+} signals are induced by an influx of calcium that is not modulated via protein kinase C and not activated by membrane depolarization. The influx pathway is also permeable to divalent cations such as Ni^{2+}, Co^{2+} and Ba^{2+}, but is blocked by La^{3+}.

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INTRODUCTION

Vascular endothelial cells are continuously exposed to flow-induced changes in shear stress. It has already been demonstrated that these flow-induced changes affect a number of endothelial cell functions, such as: (i) the synthesis of prostacyclin (Frangos, Eskin, McIntire & Ives, 1985); (ii) activation of a potassium channel (Oleson, Clapham & Davies, 1988); (iii) production of tissue plasminogen activator (Diamond, Eskins & McIntire, 1989); (iv) changes in cytoskeleton and morphology (Dewey, Bussolari, Grimbrone, Davies & Gordon, 1981; Ives, Eskin & McIntire, 1986); (v) changes in pinocytosis (Davies, Dewey, Bussolari, Gordon & Grimbone, 1984); and (vi) modulation of the response of endothelial cells to vasoactive agonists (Mo, Eskin & Schilling, 1991; for a review see Nollert, Diamond & McIntire, 1991).

Transmembrane Ca^{2+} influx via stretch-activated ion channels also occurs in endothelial cells and could be connected to Ca^{2+} signalling in these cells (Lansman, Hallam & Rink, 1987). Direct effects of shear stress on intracellular Ca^{2+} transients have been described by Ando, Komatsuda & Kamiya (1988) and by Goligorsky (1988). However, these effects were not related to the magnitude of the shear stress. They are also at variance with the findings of Mo *et al.* (1991), who did not observe direct shear stress effects on intracellular calcium.

A direct influence of mechanical events on Ca^{2+} signalling could be an intriguing explanation of the modulation of different cell functions by shear stress. In this report, we describe shear stress-induced Ca^{2+} transients in endothelial cells from human umbilical cord veins. These transients are modulated by extracellular Ca^{2+} and are obviously different from the Ca^{2+} transients induced by agonists. We propose that shear stress mediates a transmembrane influx of calcium via a pathway that is permeable to calcium and barium, but also to other divalent cations such as cobalt and nickel, which are blockers of agonist-induced Ca^{2+} entry (Jacob, 1990).

METHODS

Isolation and culture of endothelial cells

Endothelial cells were prepared from human umbilical cord veins by a collagenase digestion procedure (Type II, Sigma) as described previously (Jaffe, Nachman, Becker & Minick, 1973). The use of endothelial cells was approved by the ethical commission of the KU Leuven. Cells were grown in Medium 199 containing 1 mg ml^{-1} ATP, 10% human serum, 2 mM L-glutamine, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 2·5 μl g^{-1} amphotericin B. Cultures were maintained at 37 °C in a fully humidified atmosphere of 10% CO_2 in air. The culture medium was exchanged every 48 h. Cells were detached by exposure to 0·05% trypsin in a Ca^{2+}- and Mg^{2+}-free solution for about 3 min, reseeded on gelatine-coated cover slips, and kept in culture for 2–4 days before use. Under these conditions, cells were not confluent and single endothelial cells could be used for our experiments. We only used cells from primary culture up to the second passage.

Materials

For $[Ca^{2+}]_i$ measurements, cells were incubated with 2 μM of the acetoxymethyl ester Fura-2 AM (Molecular Probes, Eugene, OR, USA) dissolved in normal Krebs solution for 20 min at room temperature, and thereafter for another 20 min at 37 °C. The bath perfusion solution (Krebs solution) used in all experiments had the following composition (mM): 140 NaCl, 1·5 or 10 $CaCl_2$, 5·9 KCl, 1·2 $MgCl_2$, 11·5 HEPES–NaOH, 10 glucose, titrated to pH 7·3 with NaOH. $NiCl_2$, $CoCl_2$ and $BaCl_2$ were applied in concentrations between 1 and 10 mM. Histamine (Merck) was normally used in concentrations between 1 and 100 μM. In a few experiments we have used 20 and 200 nM

concentrations of this agonist. To compare the response of the cells to shear stress with that of an agonist, we incubated the cells for 10 min with 1 μM phorbol ester (12-*O*-tetradecanoyl phorbol-13-acetate, TPA, Sigma). All experiments were performed at room temperature.

Measurement of $[Ca^{2+}]_i$

After loading with Fura-2 AM, cells on cover slips were washed three times in the experimental chamber with Krebs solution to remove the extracellular Fura-2 AM. A photomultiplier-based system was used for Ca^{2+} monitoring (inverted microscope Zeiss IM 10; filter wheel, amplifier and controller, Luigs & Neumann Ratingen, Germany; photomultiplier unit, Hamamatsu, Japan). The technique used was described in detail elsewhere (Neher, 1989). In short, cells were illuminated alternately with excitation wavelengths of 360 and 390 nm via a rotating filter wheel (speed between 2 and 3 measurements s^{-1}). The intensity of the excitation light was attenuated to reduce bleaching of the dye (time constant of bleaching > 800 s). Autofluorescence was measured on cell-free parts of the cover slip, and was automatically subtracted. Apparent concentration of free calcium was calculated from the fluorescence ratio, R, according to Grynkiewicz, Poenie & Tsien (1985):

$$[Ca^{2+}]_i = K_{eff}\frac{R-R_0}{R_1-R}, \quad (1)$$

where K_{eff} is the 'effective binding constant', R_0 and R_1 represent the fluorescence ratios at zero and at high calcium. The calibration procedure was identical to that described previously by Neher (1989). K_{eff} was calculated from actual calibration values $K_{eff} = 300\ (R_1-R)/(R-R_0)$, where R is the ratio at 300 nM Ca^{2+}. For data acquisition we used an eight-channel analog-to-digital converter (Max Planck Institute of Biophysical Chemistry, Göttingen) connected to an ATARI Mega 4 computer system. At two measurements per second the analysis package allowed continuous data acquisition of approximately 30 min from the same cell.

Flow system

Under resting conditions the cells were superfused by a slow bath perfusion. Shear stress was induced by directing a stream of Krebs solution along the surface of a single endothelial cell through a multibarrelled pipette with a common opening of about 150 μm. The position of the pipette and the angle between cell and pipette were adjusted in order to obtain a non-turbulent stream of fluid which is homogeneously distributed over the whole cell surface. The flow of solution was driven by gravity and the flow rate was changed by varying the hydrostatic pressure between 0 and 75 cmH_2O. The flow of solution onto the cells was switched on and off by a system of computer-controlled valves. This method could also be used to generate a repetitive series of shear stress pulses. From the geometry of the superfusion system, shear stress τ could be estimated from (Oleson *et al.* 1988):

$$\tau = \frac{4\,\mu Q}{\pi r^3}, \quad (2)$$

where μ represents the fluid viscosity (0·007 P), Q the flow rate through the superfusion pipette (between 0 and 10^{-3} ml s^{-1}) and r the radius of the cylinder of fluid that streams out of the pipette (approximately 80 μm). The values for shear stress calculated from eqn (2) range between 0 and 50 dyn cm^{-2}, and are in the physiological range or slightly higher. The multibarrelled pipette also allowed the switch between different solutions during the experiment while maintaining a constant shear stress.

Under the following conditions reproducible Ca^{2+} transients could be evoked by shear stress with a success rate of nearly 100%: (i) cells grown on gelatine-coated cover slips, (ii) use of 10 mM Ca^{2+} in the pipette solutions, (iii) careful adjustment of the fluid stream to the geometry of the cell so that it is almost homogeneously covered by the fluid stream out of the pipette.

Statistics

Whenever possible data obtained from the same cell were compared. Otherwise means ± standard error of the mean were calculated from pooled data. For tests of significance, we used Student's paired or unpaired t test.

RESULTS

Shear stress-induced Ca^{2+} transients

Figure 1 shows a typical example of a shear stress-induced Ca^{2+} transient. A stream of fluid was blown along the surface of a single endothelial cell inducing a shear stress of approximately 25 dyn cm^{-2}. The cell was exposed to the increased shear for 60 s, during which period a Ca^{2+} transient developed with a peak value of about 530 nM. After switching off the flow, the Ca^{2+} signal decreased slowly to near basal levels with a time constant of 84 s.

From twenty-five cells exposed to shear stresses between 20 and 30 dyn cm^{-2} we measured a decay time constant of $82{\cdot}3 \pm 17{\cdot}8$ s. This recovery was much slower than the decay of the Ca^{2+} signals evoked by 100 μM histamine, which had time constants in the range 25–35 s. The peak value of the Ca^{2+} transients induced by shear stress calculated from the same twenty-five cells amounted to 587 ± 88 nM.

The pattern of the response of the endothelial cells to shear stress was variable. Figure 2 shows two of the most frequently observed patterns. In about 60% of the cells we observed a Ca^{2+} transient as shown in Figs 1 and 2*A*. More than 30% of the cells responded with a small rise in intracellular Ca^{2+} during the shear stress pulse, which further increased to a peak after switching off the shear stress (Fig. 2*B*). This signal resembles a Ca^{2+}-gated response with a flat Ca^{2+} increase that continues into a steep onset of intracellular calcium rise. Most of the remaining cells showed no detectable $[Ca^{2+}]_i$ increase during the shear stress pulse, but showed a distinct peak after switching off the flow of solution onto the cells. A few cells showed Ca^{2+} transients similar to agonist-induced Ca^{2+} responses with a fast onset immediately after switching on the shear stress.

The amplitude of the shear stress-induced Ca^{2+} transients was strongly dependent on the flow rate. Figure 3*A* shows responses to various shear stresses obtained in the same cell induced by different flow rates. If the hydrostatic pressure was increased from 20 (control) to 75 cmH_2O (first response), $[Ca^{2+}]_i$ increased from 51 to 498 nM. At 45 cmH_2O (second response) and at 60 cmH_2O (third response) $[Ca^{2+}]_i$ increased to 148 and 341 nM respectively. The subsequent shear stress pulse at 35 cmH_2O did not evoke a detectable increase in $[Ca^{2+}]_i$. These hydrostatic pressures corresponded to shear stresses of 52, 41, 31, 24 and 14 (control) dyn cm^{-2}. In order to compare the responses obtained in different cells, we have normalized the Ca^{2+} transients to the maximally observed responses at high shear stress in that cell. The average values of these normalized responses obtained from various cells are represented as a function of the applied shear stress in Fig. 3*B*. From these data we have calculated that the half-maximal increase in $[Ca^{2+}]_i$ occurred at a shear stress of 30·2 dyn cm^{-2}.

Dependence of the shear stress response on extracellular calcium

Shear stress responses also critically depended on the calcium concentration in the pipette solution with which the cell was superfused. If in the same trial the Ca^{2+} concentration in the pipette solution was changed between 1·5 and 10 mM the peak of the shear stress-induced intracellular Ca^{2+} transient was increased (Fig. 4). Its value increased from 246 ± 48 to 1125 ± 151 nM ($n = 5$ cells). Moreover, the shear stress response was completely abolished in Ca^{2+}-free solution. With 10 mM Ca^{2+} in

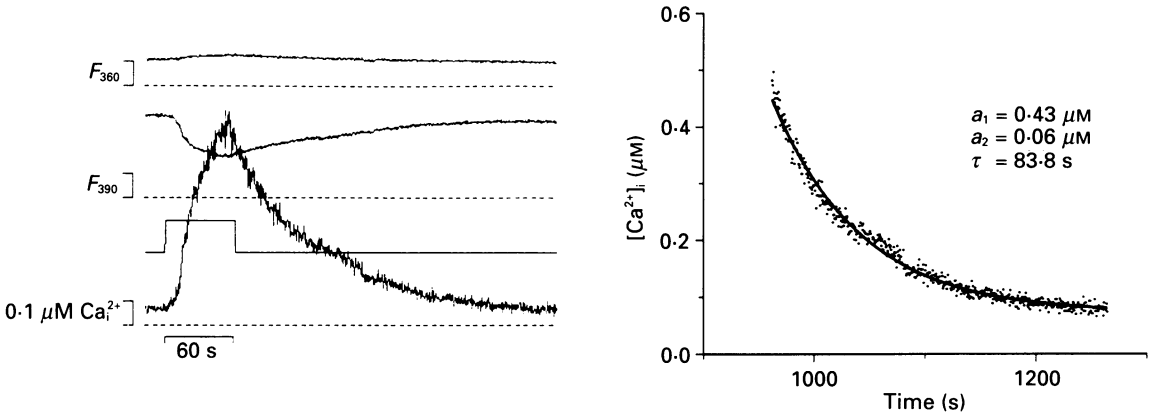

Fig. 1. Shear stress-induced Ca^{2+} transients in endothelial cells from umbilical cord vein. A stream of Krebs solution was directed for 60 s along the surface of a single endothelial cell, as indicated by the rectangular pulse in the middle panel. The upper two traces show the fluorescence signals (F) measured at 360 and 390 nm (in arbitrary units). $[Ca^{2+}]_i$ was calculated according to eqn (1), and is represented in the lower trace. In the right-hand panel the decay of the Ca^{2+} signal, represented by the symbols, is fitted by a single exponential ($[Ca^{2+}]_i = a_1 \exp(-t/\tau) + a_2$), where t is the time starting from the peak of the Ca^{2+} transient, $a_1 + a_2$ is the peak Ca^{2+} value during the shear stress in μm, a_2 is the resting Ca^{2+} level in μM.

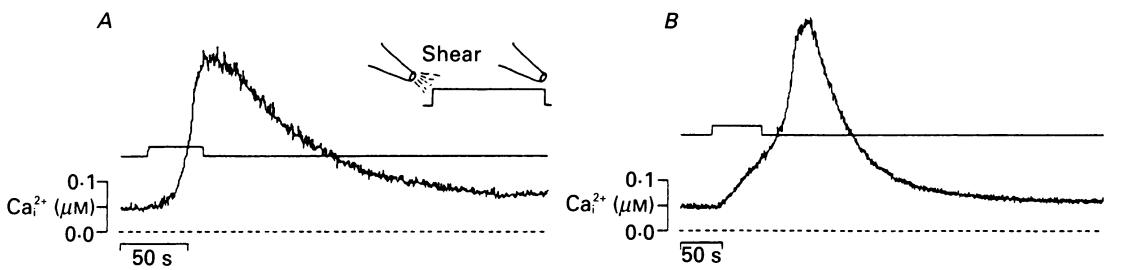

Fig. 2. Different patterns of shear stress-induced Ca^{2+} responses. Panel A shows the most frequently observed pattern characterized by a fast increase in $[Ca^{2+}]_i$ during the streaming pulse, as indicated by the rectangular trace, and a slow decline after switching off the shear stress pulse. Panel B represents another observed pattern. $[Ca^{2+}]_i$ increases slowly and moderately during the shear stress pulse, but rises to a much higher level after switching off the shear stress.

the pipette solution, shear stress-induced transients could be evoked in almost every cell.

Not all cells responded to a 30 or 60 s shear stress pulse at physiological Ca^{2+} concentrations (1·5 mM Ca^{2+}). However, at this concentration Ca^{2+} transients could always be cumulatively evoked. If short stream pulses were repetitively applied, summation of the Ca^{2+} signals could be observed (Fig. 5). This response might reflect the physiological situation of a pulsatile changing shear stress in the blood vessel.

The hypothesis that the shear stress-evoked Ca^{2+} transients are due to influx of extracellular Ca^{2+} was further tested by modifying the driving force for calcium ions.

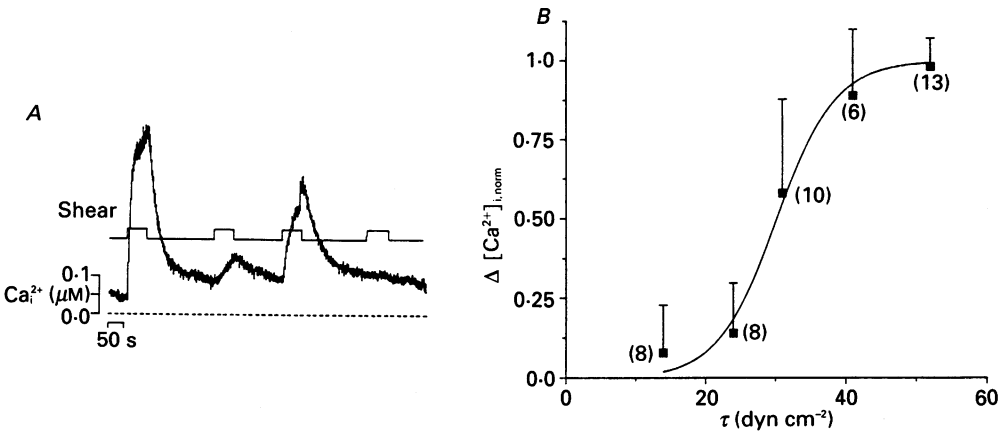

Fig. 3. The shear stress response can be graded. The original traces in *A* show the intracellular Ca^{2+} transients evoked by different flow rates at hydrostatic pressures of 75, 45, 60 and 35 cmH_2O respectively. Panel *B* summarizes the data obtained in various cells. The normalized peak increases in $[Ca^{2+}]_i$ ($\Delta [Ca^{2+}]_{i,norm}$) are plotted as a function of the applied shear stress, calculated according to eqn (2). Half-maximal activation ($\tau_{\frac{1}{2}}$) occurred at a shear stress of 30·2 dyn cm^{-2}.

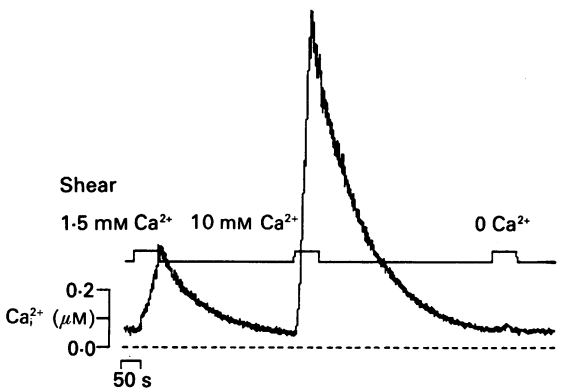

Fig. 4. The shear stress response depends on the extracellular Ca^{2+} concentration. Ca^{2+} transients were measured in the same cell perfused with solutions of different Ca^{2+} concentrations at the same flow rate (hydrostatic pressure of 75 cmH_2O, corresponding to a shear stress of 52 dyn cm^{-2}). Shear stress pulses of 60 s duration were applied from a multibarrelled pipette containing these different solutions.

We have therefore depolarized the cells by increasing the extracellular K^+ concentration. This resulted, in about 40% of the cells, in a reduction of the Ca^{2+} transient (Fig. 6*A*), but did not affect the shear stress response in the remaining 60% of the cells. This variability may be related to the variable resting potential of endothelial cells, as reported by Mehrke & Daut (1990). Obviously, strong effects would only be expected if the resting potential were negative and close to the potassium equilibrium potential.

Further evidence for the hypothesis that shear stress might activate a

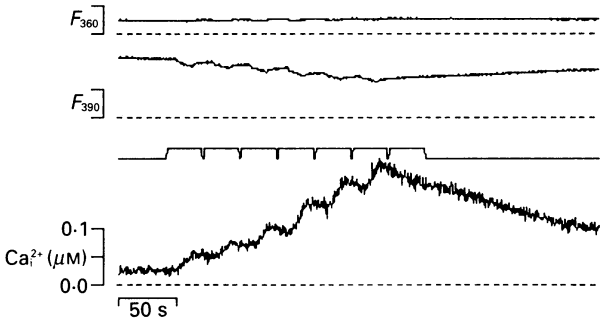

Fig. 5. Summation of the intracellular Ca^{2+} signals by repetitive shear stress pulses. Repetitive shear stress pulses of 30 s duration were applied every 5 s using a pipette solution containing 1·5 mM Ca^{2+}. The upper traces show the original fluorescence signals at 360 and 390 nm; the bottom trace represents the calculated Ca^{2+} signal.

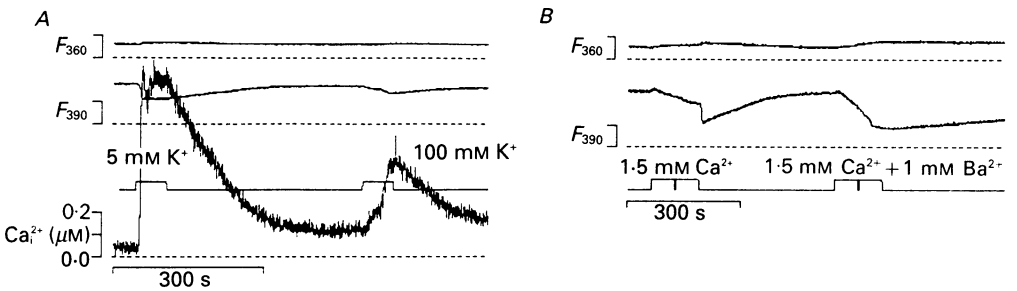

Fig. 6. *A*, Ca^{2+} transients induced by shear stress pulses of the same magnitude using pipette solutions containing either 5 or 100 mM K^+. *B*, Ca^{2+} transients induced by shear stress pulses of similar magnitude using pipette solutions containing either 1·5 mM $CaCl_2$ or 1·5 mM $CaCl_2$ + 1 mM $BaCl_2$. The shear stress responses were evoked by a double shear stress pulse. The upper two traces in each panel show the original signals at 360 and 390 nm. The lower trace in panel *A* shows the calculated Ca^{2+} response. The decrease of the 390 nm signal in panel *B* indicates that Ca^{2+} and/or Ba^{2+} enter the cell during shear stress. The different solutions were applied at the same flow rate through a multibarrelled pipette with a common opening.

transmembrane entry of divalent cations was obtained from experiments in which other divalent cations were used. In the presence of Ba^{2+} we observed drastically increased fluorescence signals, as shown in Fig. 6*B*.

Comparison of shear stress- and agonist-induced Ca^{2+} transients

Quenching experiments with manganese (Jacob, 1990) have shown that nickel blocks a putative agonist-activated pathway for Ca^{2+} entry. Therefore we have also investigated the effects of Ni^{2+} on the shear stress-induced Ca^{2+} transients. If 10 mM calcium was substituted by 10 mM nickel, we could observe changes in the intracellular fluorescence when shear stress was applied (Fig. 7*A*), but not in the absence of shear stress (not shown). This finding suggests that Ni^{2+} is entering the endothelial cell during increased shear stress presumably via the same pathway as calcium ions. Similar shear stress-activated changes in fluorescence have also been obtained when calcium was replaced by cobalt (Fig. 7*B*). The fluorescence signals do

not recover after removal of the shear stress, which is probably due to the absence of a sequestration mechanism for these divalent cations. On the other hand, if lanthanum at a concentration of 1 mM is used in the presence of 10 mM Ca^{2+}, shear stress responses were completely and reversibly suppressed (Fig. 7*C*). Also the shear

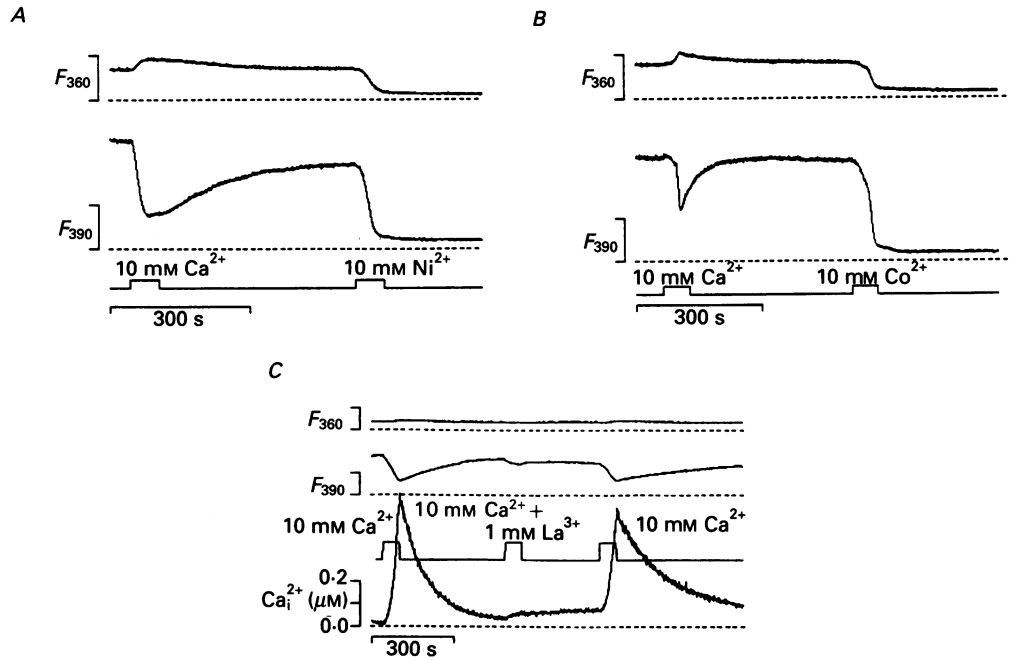

Fig. 7. Shear stress-induced Ca^{2+} transients after substitution of 10 mM Ca^{2+} by 10 mM Ni^{2+} (*A*) or 10 mM Co^{2+} (*B*). Under both conditions there was a marked decrease of the fluorescence signals at 360 and 390 nm, indicating that both divalent cations can enter the cells by a shear stress-activated pathway. Panel *C* shows that the Ca^{2+} transients induced by shear stress in the presence of 10 mM Ca^{2+} are largely and reversibly attenuated in the presence of 1 mM La^{3+}. Calibration bars of fluorescence signals are in arbitrary units.

stress-evoked quenching of the Fura-2 fluorescence in the presence of other divalent cations was largely abolished in the presence of 1 mM La^{3+} (not shown).

It is well known that vasoactive agonists induce a transient increase in the intracellular Ca^{2+} concentration of endothelial cells. Figure 8*A* shows Ca^{2+} transients induced by different concentrations of histamine. This response can be completely blocked by a short pre-incubation of endothelial cells in a solution containing 1 μM of phorbol ester (TPA) that is known to activate the protein kinase C (for other agonists see also Heller, Bussolino, Ghigo, Garbarino, Schröder, Pescarmona, Till & Bosia, 1991). In the same cells in which the response to histamine was completely blocked by TPA we still could induce shear stress-dependent Ca^{2+} transients (Fig. 8*B*). This finding also points to distinct Ca^{2+} entry mechanisms activated by agonists and by shear stress. The lack of a TPA effect on the response to shear stress indicates that this Ca^{2+} entry pathway is not modulated via a protein kinase C-sensitive mechanism.

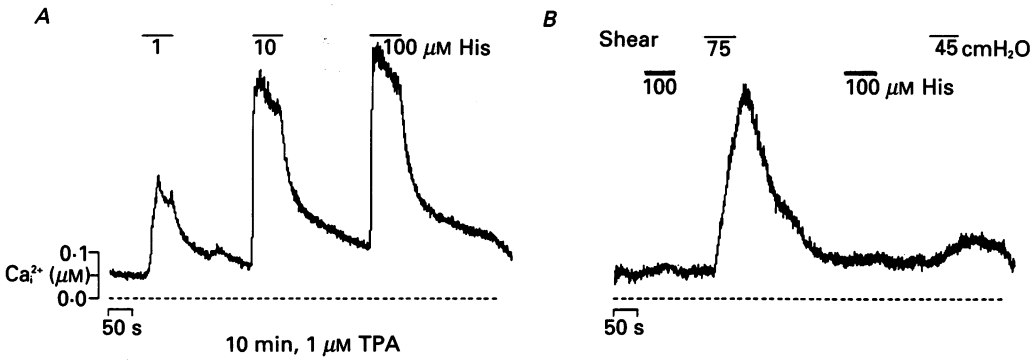

Fig. 8. Effect of TPA on the agonist-induced and on the shear stress-activated Ca^{2+} transients. Panel *A* shows Ca^{2+} transients induced by different histamine (His) concentrations. This same cell was thereafter incubated for 10 min with 1 μM of the phorbol ester TPA. The subsequent response to stimulation with 100 μM histamine (lower trace) does not evoke any Ca^{2+} response. However the cell still responds to the application of shear stress. The bars indicate the application of histamine or of shear stress.

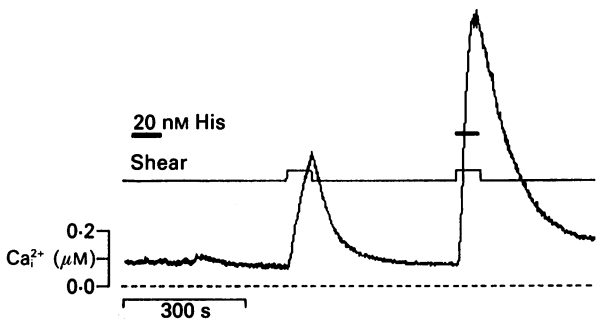

Fig. 9. Shear stress sensitizes the action of histamine. In the absence of shear stress 20 nM histamine (horizontal bar) did not evoke a Ca^{2+} transient. Application of shear stress (hydrostatic pressure of 45 cmH_2O), as indicated by the rectangular pulse, evoked a small Ca^{2+} transient. Application of both 20 nM histamine and shear stress evoked a much larger Ca^{2+} transient.

Shear stress potentiates agonist-induced Ca^{2+} transients

In endothelial cells in which shear stress is minimized, the threshold for histamine-induced responses was about 1 μM. In the presence of a submaximal shear stress, we could, however, evoke histamine-induced Ca^{2+} transients at concentrations as low as 20 nM (Fig. 9). The responses to 20 and 200 nM histamine in the presence of a submaximal shear stress were 32 ($n = 3$) and 54% ($n = 4$) of the maximal responses at 100 μM without shear stress, respectively.

DISCUSSION

Many physiological functions of vascular endothelial cells are affected by shear stress. It is still unknown how this mechanical event is linked to the cellular response. The simplest explanation would be a direct influence on Ca^{2+} signalling, as already

suggested by Ando *et al.* (1988). However, in contrast with our observations, the Ca^{2+} transients reported by these authors were not dependent on the magnitude of the applied shear stress. On the other hand, Mo *et al.* (1991) did not observe direct effects of shear stress on the intracellular Ca^{2+} concentration, but observed a striking potentiation of the ATP-induced Ca^{2+} transients by shear stress. This effect was explained by assuming that the concentration of ATP at the cell surface is dependent on the flow rate, because ATP is continuously degraded by a high ectonucleotidase activity at the cell surface. Our results are at variance with both reports, and show a graded response to shear stress and a potentiation of the histamine responses. A possible explanation for the absence of a graded shear stress response in these reports might be different experimental conditions, because we have increased the Ca^{2+} concentration in the perfusion solutions to 10 mM in order to obtain reproducible shear stress responses. Also the cell culture conditions in our experiments are slightly different. We have used Medium 199 for cell growth, which contains 1 mg ml^{-1} ATP, whereas Mo *et al.* (1991) used an ATP-free Dulbecco's modified Eagle's medium. It is not clear how the presence of a micromolar concentration of ATP in the medium, which probably matches the normal physiological situation (Coade & Pearson, 1989), could explain these divergent results. Even if the different culture conditions might reflect different cell properties, there is no doubt about a direct modulation of the intracellular calcium concentration by shear stress in the cells we have used.

Reproducible shear stress-dependent Ca^{2+} responses could be evoked in nearly 100% of the cells under the following conditions: (i) cells grown on gelatine-coated cover slips, (ii) use of 10 mM Ca^{2+} in the pipette solution to induce shear stress, (iii) careful adjustment of the flow to the cell geometry. These responses were graded and depended on the extracellular Ca^{2+} concentration. Our data hint at a possible Ca^{2+} influx activated by shear stress. A stretch-activated channel, with a higher permeability for calcium ions than for monovalent cations, has been described in vascular endothelial cells by Lansman *et al.* (1987). These channels are activated without any significant delay when mechanical stress is applied, whereas the Ca^{2+} transients in our experiments are delayed with respect to the application of shear stress. This rather large and variable delay indicates that the latter responses may be mediated by a second messenger generated by shear stress rather than by a direct activation of a Ca^{2+} influx pathway. This stretch-activated channel is also different from the non-selective cation channel that has a much higher permeability for monovalent cations than for calcium (Nilius, 1990), and which is probably activated by agonists. Our experimental data also point to some striking differences between the Ca^{2+} transients induced by shear stress and by agonists. Agonist-induced Ca^{2+} entry can be blocked by divalent cations, such as Ni^{2+} (Jacob, 1990), but these cations enter the cell via the shear stress-activated pathway. The shear stress-induced Ca^{2+} transient was not affected by phorbol esters, whereas the agonist-induced response was completely inhibited. The decay of the mechanically evoked signals was slower than the decrease in intracellular Ca^{2+} after stimulation with histamine. One possible explanation of the acceleration of the decay of agonist-induced signals might be the concomitant activation of protein kinase C via diacyl glycerol that would decrease intracellular calcium. Such an effect would not be activated by the mechanical response. The onset of the increase in intracellular calcium induced by shear stress

was in most cells much slower than that during agonist stimulation. Recently, we have recorded a shear stress-induced inward current in single endothelial cells which matches the time course of the observed Ca^{2+} transients. This current reverses near 0 mV and is about 50 pA at −40 mV (B. Nilius & G. Droogmans, unpublished observation). However, a clear cut evaluation of the influx mechanism, presumably via a mechanically activated ion channel, is still lacking.

In accordance with the findings of Mo *et al.* (1991), we have also observed a sensitization of the agonist response by shear stress. If one considers that intracellular calcium can also potentiate the actions of inositol 1,4,5-trisphosphate at its receptor (Bezprozvanny, Watras & Ehrlich, 1991; Finch, Turner & Goldin, 1991), a sensitizing action of shear stress-mediated Ca^{2+} influx on agonist responses would be expected.

Our data can explain effects of haemodynamically generated shear stress forces by a direct influence on the intracellular Ca^{2+} concentration. The shear stress applied in some of our experiments might be higher than the physiological values, but it is clear that repetitively applied small shear stress pulses can modulate the intracellular calcium concentration and also that physiological shear stresses can potentiate the action of circulating agents. Such a direct modulation would link mechanical events to intracellular signals.

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