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Intracellular Calcium Changes in Rat Aortic Smooth Muscle Cells in Response to Fluid Flow

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Abstract

Vascular smooth muscle cells (VSM) are normally exposed to transmural fluid flow shear stresses, and after vascular injury, blood flow shear stresses are imposed upon them. Since Ca^{2+} is a ubiquitous intracellular signaling molecule, we examined the effects of fluid flow on intracellular Ca^{2+} concentration in rat aortic smooth muscle cells to assess VSM responsiveness to shear stress. Cells loaded with fura 2 were exposed to steady flow shear stress levels of 0.5–10.0 dyn/cm² in a parallel-plate flow chamber. The percentage of cells displaying a rise in cytosolic Ca^{2+} ion concentration ($[\text{Ca}^{2+}]_i$) increased in response to increasing flow, but there was no effect of flow on the ($[\text{Ca}^{2+}]_i$) amplitude of responding cells. Addition of Gd^{3+} (10 μM) or thapsigargin (50 nM) significantly reduced the percentage of cells responding and the response amplitude, suggesting that influx of Ca^{2+} through ion channels and release from intracellular stores contribute to the rise in ($[\text{Ca}^{2+}]_i$) in response to flow. The addition of nifedipine (1 or 10 μM) or ryanodine (10 μM) also significantly reduced the response amplitude, further defining the role of ion channels and intracellular stores in the Ca^{2+} response.

Keywords

Shear stress; Smooth muscle cells; Intracellular Ca^{2+} ; Stretch-activated channels; Myogenic tone

INTRODUCTION

Vascular smooth muscle (VSM) cells are continuously exposed to solid mechanical stretching forces associated with the hoop stresses that are driven by the vascular pressure pulse. There have been several reports demonstrating that smooth muscle cells (SMCs) are responsive to mechanical stretching forces *in vitro*.^{4,8,29} Little research, however, has been directed at VSM response to forces generated by blood flow shear stress. Since the VSM

cells lie underneath the endothelial layer, they are not normally exposed directly to mechanical shear stress associated with blood flow.⁶ However, after the endothelium and internal elastic lamina (IEL) are disrupted by vascular procedures such as angioplasty or in the anastomotic region of vascular grafts, VSM cells are directly exposed to blood flow and their function may be modulated by changes in the local hemodynamic environment.³² Experiments in animal models of atherosclerosis and intimal hyperplasia have shown that luminal SMCs are present from days to months.^{5,14} In these situations where the endothelium is injured, arterial levels of blood flow wall shear stresses, on the order of 10–20 dyn/cm², may be imposed on the superficial VSM. On the other hand, healthy VSM cells, beneath the intact endothelium, are also exposed to shear stress via transmural flow driven by the transmural pressure gradient.³⁹ Wall shear stresses, associated with transmural interstitial flow on the order of 1–3 dyn/cm² have been predicted at transmural pressure gradients of 70–180 mmHg.³⁹ The most superficial layer of SMCs, right beneath the IEL, may be exposed to even higher levels of shear stress due to the funneling of flow through fenestrae in the IEL.³⁷ *In vitro* studies of flow through VSM-laden collagen gels, simulating interstitial flow through the arterial wall, have shown that low levels of interstitial flow can stimulate VSM cell biochemical production.⁴⁰ Shear stress on VSM driven by transmural pressure is elevated in direct proportion to vascular pressure and by increases in blood flow shear stress which increase the hydraulic conductivity of the endothelial layer.^{30,35} Alshihabi *et al.*¹ demonstrated that rat aortic smooth muscle cells (RASMCs) in culture produced prostacyclin at a significant rate in response to shear stress of only 0.5 dyn/cm² and the rate of production was no higher at 20 dyn/cm². Papadaki *et al.*³² demonstrated that exposure of cultured human aortic smooth muscle cells to flow resulted in nitric oxide production with no difference in nitrite levels among the different shear stress levels (1.1, 5, and 25 dyn/cm²). Sterpetti *et al.*³⁶ studied the effects of laminar flow (6 dyn/cm²) on the proliferation rate and morphology of bovine aortic smooth muscle cells in culture. They found that shear stress decreased the proliferation rate of smooth muscle cells and tended to align them in the direction of flow.

Ca²⁺ is an important second messenger mediating many cellular biomolecular responses. Indeed, fluid flow has been shown to modulate Ca²⁺ dynamics in bone cells, endothelial cells, and chondrocytes.^{15,17,19,44} Ca²⁺ may move inward across the plasma membrane through stretch-activated channels (SACs), which are present in both visceral²⁴ and vascular smooth muscle^{7,23} as well as voltage-operated channels (VOCs). In addition to Ca²⁺ entering the cell through ion channels in the plasma membrane, the release of Ca²⁺ from intracellular stores via second messenger systems could serve to mobilize [Ca²⁺]_i. Any one, or a combination, of these mechanisms may affect smooth muscle cell behavior.

We examined the effect of fluid flow on [Ca²⁺]_i in RASMCs using a parallel-plate flow chamber and fura 2 to image the cells. The mobilization of [Ca²⁺]_i by fluid flow was assessed in terms of potential mechanisms that might regulate [Ca²⁺]_i signaling. A pharmacological approach was used to characterize the role of SACs and VOCs relative to intracellular release mechanisms. We conclude that unrelated mechanisms of Ca²⁺ influx and internal release are present and both are significant in the dynamics of [Ca²⁺]_i signaling.

MATERIALS AND METHODS

All chemicals were purchased from Sigma unless otherwise noted.

Smooth Muscle Cell Isolation and Culture

RASMCs were enzymatically isolated from the thoracic aortas of adult Sprague-Dawley rats following euthanization by carbon dioxide asphyxiation by procedures approved by the Institutional Animal Care and Use Committee. The isolation procedures have been described previously.¹ The cells were cultured at a density of 2×10^4 cells/cm² in Dulbecco's minimal essential medium (DMEM/F12) with 50 U/ml penicillin and 0.05 mg/ml streptomycin (1% P/S) supplemented with 10% fetal bovine serum (FBS). Cells were positively identified by their characteristic hill-and-valley growth pattern, and their positive staining with monoclonal anti- α -smooth muscle actin. Cells to be used for experiments were grown to confluency (4 days) in DMEM/F12 + 1% P/S supplemented with 10% FBS. These subcultured cells continued to stain positively for α -actin. The cells were then plated onto 75×35- and 1 to 1.7-mm-thick quartz slides (Friedrich & Dimmock, Millville, NJ) for the flow experiments at a density of 80,000 cells/slide for two days. Only passages 3–6 were used for experiments. All of the experiments in this study were based on cells from two primary cultures with three rats per primary.

Fluid Flow—The parallel-plate flow chamber was a modification of the design of Frangos *et al.*¹² The quartz slide with attached RASMCs formed the bottom plate of the flow chamber and a polycarbonate plate formed the top. A silastic gasket (SF Medical, Hudson, MA) was used to maintain a uniform gap of 280 or 232 μ m between the two parallel plates. All three components were held together by a vacuum that was applied through a side port and maintained at the periphery of the slide in a machine-milled groove in the polycarbonate plate, overlaid by the gasket that was perforated with 2-mm-diam holes. This formed a sealed, 38×10 or 24×60 mm flow channel with a parallel-plate geometry. Tyrode's solution with or without 2% FBS entered the chamber through an inlet port, passed into the channel via an 8×1 or 15×1 mm slit, flowed across the cell layer, and exited via an identical outlet slit and second side port. Fluid flow was generated with a Harvard syringe pump (Harvard Apparatus, Natick, MA), which induced shear stresses of 10.0, 5.0, 2.3, 1.1, and 0.5 dyn/cm². Wall shear stress was calculated, assuming fully developed laminar flow between infinite parallel plates, using the following equation: $\tau = 6 \mu Q / bh^2$ where τ is the wall shear stress, μ is the viscosity, Q is the fluid flow rate, b is the width of the flow channel, and h is the height of the flow channel.²⁴ These shear stresses covered a range that would be encountered by superficial VSM cells exposed to blood flow in a denuded artery (10 dyn/cm²), or VSM cells exposed to transmural flow in a normal artery (1 dyn/cm²).

Ca²⁺ Imaging—Preconfluent cells on quartz slides were washed with Tyrode's solution and then incubated with 1 μ M fura 2-acetoxymethyl ester (Molecular Probes, Eugene, OR) solution (in Tyrode's) for 30 min at 37 °C. The cells were then washed with fresh Tyrode's solution at 37 °C, and the slide was mounted on a parallel-plate chamber. The chamber was placed on an inverted fluorescence microscope (Nikon Diaphot 300 or Olympus IMT-2) and left undisturbed for 30 min. Fura-2-loaded cells were excited with UV light filtered at 340

and 380 nm alternately. Emitted light passed through the objective and a 510 nm filter and was detected by an intensified charge-coupled-device camera (Videoscope, Sterling, VA, or Dage MTI, Inc. Michigan City, IN). Data were sampled and recorded every 4 s using the Metafluor (Universal Imaging Co., Downingtown, PA) or Axon (Axon Instruments, Inc., Union City, CA) imaging system. A calibration curve was used to convert the 340:380 ratio values from individual cells into $[Ca^{2+}]_i$. This was done by acquiring 340:380 values (background subtracted) for a series of solutions of known free Ca^{2+} concentration (0–39.8 μ M, Molecular Probes) and 1 μ M fura 2 pentapotassium salt (Molecular Probes).¹⁶

Data Analysis—Data were collected for 1 min at the start of each experiment in the absence of flow to establish a steady base line $[Ca^{2+}]_i$ for each cell and then for an additional 3 min after the onset of flow. To identify Ca^{2+} transients we used an adapted numerical procedure from mechanical fatigue analysis, known as rainflow cycle counting.⁹ This simple algorithm reliably and automatically identifies and determines the amplitudes of spikes and transients in time history data in the presence of background noise. We have previously used this algorithm to identify transients in $[Ca^{2+}]_i$ in bone cells.²² Other Ca^{2+} transients were determined by calibration algorithms, using the Axon Imaging Workbench software program.

The “basal normalized amplitude” of a calcium response is defined as the maximum value of $[Ca^{2+}]_i$ during the 3 min flow period minus the average value of $[Ca^{2+}]_i$ during the 1 min preflow period (the amplitude), divided by the average no-flow value of $[Ca^{2+}]_i$. No-flow control experiments were carried out in which cells were placed on a coverslip but did not experience shear for 30 min. The “control normalized amplitude” of a calcium response is defined as the 10 dyn/cm² step response amplitude in the presence of Ca^{2+} mobilization inhibitors divided by the amplitude in the absence of such inhibitors. We selected a 30 nM amplitude as a conservative criterion to differentiate cells exhibiting a calcium response from noise. Therefore, a cell was considered responsive if the amplitude of the calcium response exceeded 30 nM.

The pooled data presented take the form of “percentage of cells responding” (Figs. 3 and 5) and the “normalized amplitude” of response (Figs. 4, 6, and 7). Data from four to nine slides (each containing 20–45 cells) were used to determine the responses, which are presented as mean $\pm$ standard error of the mean (SEM) values. To compare observations from more than two groups, a one-way analysis of variance was used with a freedom contrast of 3 degrees. Statistical significance was calculated by analysis of SEM, mean, and *n*, using the Newman Keuls multiple comparison test. *P* < 0.05 is considered statistically significant.

RESULTS

Effect of Flow on $[Ca^{2+}]_i$

For all flow experiments, data were collected for 1 min in the absence of flow to establish steady base-line $[Ca^{2+}]_i$ levels for each cell, and then fluid flow was applied for 3 min. As a control, cells were mounted on the flow chamber but subjected to no flow for 4 min. When serum-free media was incorporated in the flow loop, there was no calcium response (data not shown). All of the remaining results described in this section were obtained with

Tyrode's solution containing 2% FBS in the flow loop. As representative data, Fig. 1 shows the effect of 10 dyn/cm² of steady shear stress on [Ca²⁺]_i in four RASMCs. All four cells responded within 10–15 s of initiating the flow. In general, the [Ca²⁺]_i remained elevated above the preflow base line beyond the 3 min duration of the flow exposure. The mean baseline [Ca²⁺]_i level before flow was 37.9 ± 2.71 nM. The basal calcium levels and fluid flow response amplitudes (e.g., Fig. 1) are similar to observations reported for endothelial cells¹⁰ and chondrocytes.⁴⁴

Cellular [Ca²⁺]_i response following successive step changes in flow may provide insight into the dynamic adaptation of VSM cells to fluid shear and the possible role of fluid phase mass transfer in the process. Figure 2 shows the [Ca²⁺]_i response to a sequence of step changes from 0 to 1.1 to 10 to 0 dyn/cm². The steps from 0 to 1.1 dyn/cm² and from 1.1 to 10 dyn/cm² each displayed a characteristic amplitude spike followed by a return toward an elevated steady-state level. The turn off from 10 to 0 dyn/cm², however, did not display a significant response.

We also determined the percentage of cells responding to flow as a function of shear stress: 7% ± 3% of control cells showed spontaneous intracellular (IC) Ca²⁺ transients in the absence of flow (control), while the percentage of cells responding increased with increasing shear stress (Fig. 3). Figure 3 suggests that the IC₅₀ threshold value for shear stress stimulation is between 0.5 and 1.1 dyn/cm². It is also apparent that the response percentages above 2.3 dyn/cm² are saturated.

While the mean percentage of cells responding increased significantly with increasing shear stress, there was no significant difference between experimental groups with regard to normalized amplitude response (Fig. 4).

Possible Role of Plasma Membrane Ion Channels in the Cellular Response to Fluid Flow—Experiments were performed in the presence of gadolinium chloride (Gd) and nifedipine (Nif) to investigate a possible role for stretch-activated channels in the fluid flow-induced intracellular Ca²⁺ response.

Gadolinium chloride has been shown to block stretch-activated channels in other cell types.^{7,8} A concentration of 10 μM Gd was used in the experimental solution (Tyrode's solution with 2% FBS). Cells were then subjected to a steady shear stress of 10 dyn/cm², in the presence or absence of extracellular Gd. In the presence of Gd, the percentage of cells responding to a shear stress of 10 dyn/cm² was significantly reduced (Fig. 5). Addition of Gd also significantly reduced the control normalized peak amplitude (Fig. 6).

A previous study indicated that Ca²⁺ entry through voltage-gated L-type channels can be reduced by nifedipine levels of 0.1 and 10 μM.³³ Concentrations of 1 and 10 μM nifedipine were used in our assays to assess the involvement of L-type VOCs. A significant decrease in control normalized peak [Ca²⁺]_i response was measured when nifedipine incubated RASMCs (60 min prior to flow onset) were subjected to 10 dyn/cm² of shear stress. In addition, the cells treated with nifedipine (1 or 10 μM) exhibited a statistically significant

dose response (Fig. 7). These results indicate the potential role of L-type VOCs in mediating RASMC $[Ca^{2+}]_i$ response to shear stress.

Role of Intracellular Ca^{2+} Stores in the Cellular Response to Fluid Flow—

Experiments were completed with thapsigargin (Thps) and ryanodine (Ryan) to explore the possible role of intracellular Ca^{2+} stores in the fluid flow-induced intracellular Ca^{2+} response. Thapsigargin is an inhibitor of the sarcoplasmic reticulum Ca^{2+} -ATP-ase pump (SERCA) and 50 nM Thps has been shown to deplete calcium stores.²¹ Ryanodine exhausts Ryan sensitive Ca^{2+} stores.⁴²

In these experiments Thps was added to the experimental solution to produce a concentration of 50 nM. The mean base-line $[Ca^{2+}]_i$ level before flow, but in the presence of 50 nM Thps, was 119 nM. This represents a significant elevation of the basal Ca^{2+} level relative to cells without the drug treatment (38 nM). Even though the basal Ca^{2+} level is elevated in the presence of Thps, a responding cell is defined as one with a calcium transient more than 30 nM above the preshear base-line level. In the presence of Thps, the percentage of cells responding to a flow rate of 10 dyn/cm² was significantly reduced (Fig. 5). Addition of Thps also significantly reduced the control normalized amplitude response (Fig. 6).

A concentration of 10 μ M Ryan elicited a reduction in RASMC $[Ca^{2+}]_i$ response *ex vivo* in studies performed by Hisayama *et al.*¹⁸ Ryanodine at a concentration of 10 μ M was used in our experiments. Cells were incubated in the presence of Ryan for 5 min prior to and during fluid flow. Ryanodine treatments significantly reduced the control normalized amplitude compared to the control value (Fig. 7).

Dual Role of Plasma Membrane Ion Channels and Intracellular Ca^{2+} Stores in the Cellular Response to Fluid Flow—

Experiments were performed to observe the effect of combining drugs, 10 μ M Gd and 50 nM Thps. In the presence of both drugs, the percentage of cells responding to a shear stress of 10 dyn/cm² was reduced significantly (Fig. 5). The response to Gd and Thps together was less than either drug alone and the reduction relative to Thps alone was statistically significant. With both Gd and Thps, the control normalized amplitude response was also decreased by more than either drug alone (Fig. 6).

DISCUSSION

Previous studies involving smooth muscle have documented responses to mechanical stress including gene expression and signal transduction.²⁸ Concerning fluid flow or shear stress specifically, Papadaki *et al.*³² showed stimulation of neuronal NOS isoform in human VSM cells at a shear stress as low as 1.1 dyn/cm². Wagner *et al.*,³⁸ in RASMCs, observed the induction of HO-1 gene expression and CO production at shear stresses of 5 dyn/cm². Sterpetti *et al.*³⁶ found that shear decreased the proliferation rate of smooth muscle cells. While a number of experiments have shown that smooth muscle cells are sensitive to flow, this is the first demonstration of fluid flow-induced Ca^{2+} response in these cells.

The results of our step change experiments with a no-flow initial state show that the dynamic response of $[Ca^{2+}]_i$ is characterized by an initial overshoot (amplitude) and subsequent decay to a level which remains elevated above the preshear base line during the course of shear stress exposure (Fig. 1). When cells are further stimulated from a finite flow base line (1.1 dyn/cm²), they show a similar sensitivity of $[Ca^{2+}]_i$ to the step change in shear stress (Fig. 2). When shear stress is removed, however, $[Ca^{2+}]_i$ does not return to the preshear base line within 100 s (Fig. 2). Our results for the step change from a no-flow initial state are similar to observations reported by Helminger *et al.*¹⁷ who exposed bovine aortic endothelial cells (BAECs) in serum-containing, ATP-free media, to step changes from 3 to 70 dyn/cm². They reported an overshoot of $[Ca^{2+}]_i$ without complete return to preshear base line. They did not report step responses from a finite flow base line or after removal of shear stress.

Dull and Davies,¹⁰ on the other hand, did perform step-up and step-down experiments with BAECs, but using a serum-free, ATP-supplemented media. They observed rapid increases in $[Ca^{2+}]_i$ in response to step increases in flow from both static and finite-flow base lines, which returned to preflow levels in the presence of sustained flow. When flow was turned off during a positive transient, $[Ca^{2+}]_i$ returned rapidly to base line. Step increase in flow in serum-free media without ATP did not elicit a $[Ca^{2+}]_i$ response. Dull and Davies¹⁰ and Dull *et al.*¹¹ interpreted the rapid response of $[Ca^{2+}]_i$ to both increases and decreases in flow in terms of a mass transport mechanism whereby increases in flow increase the flux of ATP to the cell surface where it is degraded by ectonucleotidases. Our results, based on serum-containing media without added ATP, differ significantly from Dull and Davies¹⁰ in that the positive step responses of $[Ca^{2+}]_i$ do not return to basal levels, and upon removal of flow, we do not observe a rapid return to base line (Fig. 2). These differences suggest that our responses are not mediated primarily by mass transport of a soluble species in the media. Our results for step increases in flow in serum-free media without ATP are consistent with Dull and Davies:¹⁰ there is no response of $[Ca^{2+}]_i$.

Our experiments show that a step in flow from an initial no-flow state increases the percentage of RASMCs displaying a rise in $[Ca^{2+}]_i$ in a flow rate (shear stress)-dependent manner (Fig. 3). Similar results were observed in osteoblasts¹⁹ and chondrocytes.⁴⁴ In contrast to the flow-rate-dependent effect on the number of cells responding, we found no statistically significant effect of flow rate on the amplitude of the Ca^{2+} response (Fig. 4). This is in agreement with observations in endothelial cells¹⁵ and chondrocytes.⁴⁴

Patch-clamp techniques have been used to record single-channel currents in enzymatically dispersed SMCs. Using this technique, Kirber *et al.*,²³ characterized a nonspecific cation SAC in visceral (toad stomach) smooth muscle. Likewise, in vascular (porcine coronary artery) smooth muscle, Davis *et al.*⁸ identified a nonspecific cation, SAC. Setoguchi *et al.*³⁴ suggested the existence of a SAC in smooth muscle from mesenteric resistance arteries of the guinea pig, which could be inhibited by Gd. In our studies, Gd significantly reduced both the percentage of cells responding and the amplitude of $[Ca^{2+}]_i$ following fluid flow in RASMCs (Figs. 5 and 6). This finding is consistent with studies on bone cells, in which Gd was shown to cause up to a 30% inhibition of the flow-induced intracellular Ca^{2+} response,²⁰ and chondrocytes, in which Gd caused a decrease in the percentage of cells

responding and the response amplitude by 63% and 21%, respectively.⁴⁴ Although these data suggest that SAC may be a pathway through which Ca^{2+} passes to trigger the $[\text{Ca}^{2+}]_i$ rise observed in response to fluid flow, they must be interpreted with caution, since Gd has also been shown to block L-type VOCs in ventricular and atrial myocytes^{26,27} and other Ca^{2+} currents in pituitary cells³ and xenopus oocytes.⁴¹

Our data, as well as others,³³ indicate a dose-sensitive response of $[\text{Ca}^{2+}]_i$ to the drug nifedipine (Fig. 7). Unlike Gd, nifedipine is a specific blocker of L-type VOCs, which are known to be present in visceral smooth muscle.^{2,31} Because Gd affects L-type VOCs as well as SACs, however, we cannot be sure that a SAC is operative in the shear-induced increase in $[\text{Ca}^{2+}]_i$ that we observed in RASMCs.

Another possible source of Ca^{2+} is release from intracellular Ca^{2+} stores. Thapsigargin has been shown to selectively block Ca-ATPase in vascular smooth muscle cells,⁴³ thus emptying cytosolic Ca^{2+} stores. Our data demonstrate that Thps inhibits the percentage of cells responding (Fig. 5) and the amplitude of the response (Fig. 6), suggesting that intracellular Ca^{2+} stores contribute to flow-induced cytosolic Ca^{2+} mobilization. RASMCs have been shown previously to possess ryanodine-sensitive stores as well as IP_3 sensitive stores.^{13,25} Our results indicate that the $[\text{Ca}^{2+}]_i$ response to shear stress is not completely inhibited by the presence of either Ryan or Thps (Figs. 6 and 7).

We also found that by adding both Thps and Gd together to the perfusing media the percentage of cells responding (Fig. 5) and the amplitude of the response (Fig. 6) decreased more than with either drug alone. This suggests that ion channels and intracellular Ca^{2+} stores respond in parallel, and that both contribute to the modulation of intracellular Ca^{2+} in response to shear stress.

We have demonstrated that fluid flow shear stress can elevate intracellular Ca^{2+} in VSM *in vitro* and that ion channels and intracellular stores are involved in the process. Because Ca^{2+} is a ubiquitous intracellular signaling molecule, our studies suggest that fluid flow shear stress on SMCs, which has only recently been recognized as a potentially important stimulus for this cell type,³⁹ could modulate many physiological responses of SMCs. Further studies in intact blood vessels, however, are required to clearly establish the physiological relevance of fluid flow shear stress on vascular smooth muscle cells.

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FIGURE 1.

Effect of flow on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in rat aortic smooth muscle cells. Each line represents the $[Ca^{2+}]_i$ signal from an individual cell when subjected to a steady shear stress of 10 dyn/cm^2 . The arrow indicates onset of fluid flow.

FIGURE 2.

Effect of step changes in flow from 0 to 1.1 to 10 to 0 dyn/cm² shear stress on $[Ca^{2+}]_i$ in RASMCs. Graph is the average $[Ca^{2+}]_i$ response of 32 individual cells from 3 slides. Concentration was normalized with respect to the base line in the absence of flow. Arrows indicate onset of specific shear stress levels. Vertical bars represent $\pm$ SEM.

FIGURE 3.

Percentage of cells showing an intracellular Ca^{2+} response at shear stresses between 0 (control) and 10 dyn/cm^2 . 6.9% $\pm$ 2.5%, 48% $\pm$ 21%, 59% $\pm$ 14%, 84% $\pm$ 14%, 85% $\pm$ 5%, and 93% $\pm$ 2% of cells responded to shear stresses of 0, 0.5, 1.1, 2.3, 5, and 10 dyn/cm^2 , respectively. Each bar represents mean percentage of cells responding $\pm$ SEM. Each experiment was repeated on 4–6 separate slides, and 15–45 cells were imaged on each slide.

*Statistically significant difference from control (no flow), $P < 0.01$.

FIGURE 4.

Basal normalized $[Ca^{2+}]_i$ amplitude of responding cells at different shear stresses. The basal normalized $[Ca^{2+}]_i$ amplitude values were 1 ± 0.1 , 2.9 ± 0.3 , 2.1 ± 0.2 , 2.6 ± 0.2 , 2.3 ± 0.2 , and 2.6 ± 0.2 for shear stresses of 0, 0.5, 1.1, 2.3, 5, and 10 dyn/cm^2 , respectively. Each bar represents mean amplitude of responding cells $\pm$ SEM. Each experiment was repeated on 4–6 separate slides and 15–45 cells were imaged on each slide.

FIGURE 5.

Percentage of cells showing an intracellular Ca^{2+} response at no flow (control), at a shear stress of 10 dyn/cm² without drugs, and at a shear stress of 10 dyn/cm² in the presence of 10 μM Gd^{3+} , 50 nM of thapsigargin (Thps), and both 10 μM Gd^{3+} and 50 nM Thps. The percentage of cells responding for these cases were: 7 ± 3 , 93 ± 2 , 45 ± 4 , 30 ± 8 , and 20 ± 12 . Each bar represents the mean percentage of cells responding $\pm$ SEM, and each experiment was repeated on 4–6 separate slides containing 15–45 cells. *Statistically significant difference from 10 dyn/cm², $P < 0.001$.

FIGURE 6.

Control normalized $[Ca^{2+}]_i$ amplitude of responding cells at a shear stress of 10 dyn/cm² without drugs, and at a shear stress of 10 dyn/cm² in the presence of 10 μ M Gd³⁺, 50 nM Thps, and both 10 μ M Gd³⁺ and 50 nM Thps. The control normalized $[Ca^{2+}]_i$ amplitude values for these cases were: 1 ± 0.06 , 0.46 ± 0.03 , 0.54 ± 0.04 , and 0.35 ± 0.02 . Each bar represents the mean amplitude of responding cells $\pm$ SEM, and each experiment was repeated on 4–6 separate slides and 15–45 cells were imaged on each slide. *Statistically significant difference from 10 dyn/cm², $P < 0.001$.

FIGURE 7.

Control normalized $[Ca^{2+}]_i$ amplitude of responding cells at a shear stress of 10 dyn/cm² without drugs and at a shear stress of 10 dyn/cm² in the presence of 1 µM nifedipine (Nif), 10 µM Nif, and 10 µM ryanodine (Ryan). Control normalized $[Ca^{2+}]_i$ amplitude values for these cases were: 1 ± 0.05 , 0.7 ± 0.03 , 0.43 ± 0.02 , and 0.45 ± 0.02 . Each bar represents the mean amplitude of responding cells ± SEM, and each experiment was repeated on 4–9 separate slides and 5–27 cells were imaged on each slide. *Statistically significant difference from control, $P < 0.001$. * #Statistically significant difference from 1 µM Nif, $P < 0.001$.