CALCIUM-INDEPENDENT MYOGENIC CONSTRICTION DURING ARTERIOLAR OCCLUSION

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ABSTRACT

Myogenic constriction follows vascular stretch due to a step increase in hydrostatic pressure. This mechanism is widely thought to depend on a transient release of calcium; however, recent evidence also suggests the possibility of calcium-independent contraction responses. In the present investigation of the role for calcium, arteriolar diameters in the rat mesentery were monitored prior to and following downstream occlusion with a glass micropipette. In each experiment (N=4), a baseline occlusion induced a transient arteriolar stretch followed by constriction. Following release of this occlusion, the tissue was subsequently exposed to BAPTA-AM in each experiment, to chelate intracellular calcium. The presence of BAPTA-AM induced a sustained arteriolar dilation, indicating that baseline arteriolar tone is dependent on calcium. However, in a second arteriolar occlusion (in the presence of BAPTA-AM), the arteriolar response was the same as without BAPTA-AM, i.e., arteriolar diameter stretched, and then constricted, by similar percentages as observed in the baseline occlusion. These results suggest a calcium-independent component to the myogenic response induced by vascular occlusion of mesenteric arterioles.

INTRODUCTION

It is generally thought that calcium release following an increase in vascular pressure initiates an intracellular cascade that results in myogenic constriction. However, a recent study by Hill et al. [1] challenges this hypothesis. These investigators found that the steady state myogenic response was similar whether myogenic constriction was induced by a step increase in pressure that caused an initial vascular stretch, or by a ramp increase in pressure in the absence of vascular stretch. An initial transient increase in calcium was only observed in the step increase, leading to the interpretation that calcium may not be a requirement for myogenic responses.

Data from Civelek et al. [2] also suggest that smooth muscle contraction can occur by a calcium-independent pathway. In their studies, contraction was induced by an increase in shear stress on the surface of smooth muscle monolayers. Contraction occured despite no detectable change in intracellular calcium imaged with the fluorescent dye fura 2-AM. This contraction was attenuated by rho-kinase inhibitors, but not during chelation of intracellular calcium by BAPTA-AM.

In the present study, we use similar methodology to that of Civelek et al. to test the role for calcium in the in vivo myogenic response that occurs subsequent to vascular occlusion.

METHODS

Animal Preparation

Four male Wistar rats were initially anesthetized with Halothane followed by an intraperitoneal injection of 135 mg/kg thiobutabarbital (Inactin). The right carotid artery was cannulated to monitor systemic blood pressure, and the small intestine was exteriorized through a midline abdominal incision. The rat was placed on its right side on a Plexiglas board so that a selected section of mesentery could be draped over a glass coverslip glued on a hole centered in the board. The exposed intestine, except for the selected mesenteric section under study, was covered with gauze soaked in bicarbonate-buffered saline (BBS). After the board was mounted onto the stage of an inverted microscope, the mesentery and intestine were kept moist with a 2 ml/min superfusion of BBS bubbled with a 95% N_2 and 5% CO_2 gas mixture and warmed to 37 °C.

Video Microscopy

The mesentery was observed through a ×40 objective (Nikon Plan Apo, 0.95 N.A.) using a 100-W halogen light source, and brightfield images were captured with a color camera. The image was directed into a videocassette recorder and the taped image was used for playback analysis with an image grabber and image processor for measurement of vessel diameter.

Experimental Procedure

Arterioles having a baseline diameter of ~20-30 microns were occluded with a glass micropipette, controlled by micromanipulator, to initiate a myogenic response. This technique has been used previously

for the hamster cheek pouch [3], where arteriolar pressure upstream of the occluder increased by 22.5%. Arteriolar diameter was monitored ~400-500 microns upstream of the occlusion site. Following the baseline occlusion, vascular flow was restored and allowed to equilibrate prior to exposure of the tissue to 10 μM BAPTA-AM, which chelates intracellular calcium. BAPTA-AM superfusion was continued for 20 minutes prior to a second arteriolar occlusion.

RESULTS

Figure 1 demonstrates the arteriolar response to downstream vascular occlusion. In the first few seconds, the arteriole stretches due to the increase in hydrostatic pressure, which we have measured in separate experiments to be ~ 7 mmHg (from a baseline of 40-45 mmHg). This stretch is followed by constriction that returns arteriolar diameter toward its baseline value. Also shown in Figure 1, BAPTA-AM induces an arteriolar dilation consistent with a calcium-dependency of basal diameter. However, the transient myogenic response of the arteriole appears to be independent of calcium, in that similar constriction follows the stretch caused by a subsequent downstream occlusion. Diameters normalized to pre-occlusion values are given in Figure 2.

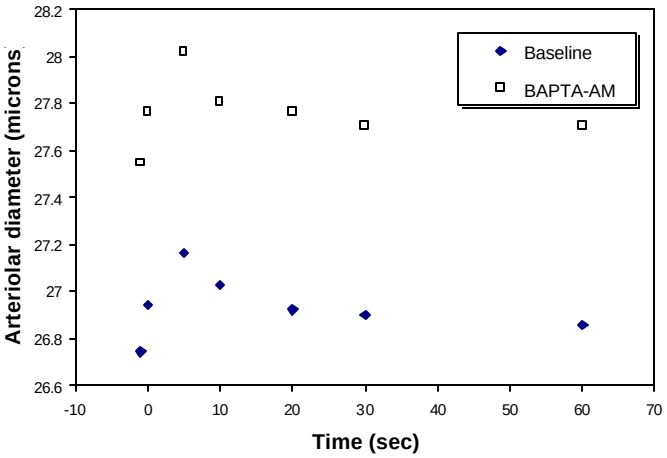

Figure 1. Arteriolar diameter during downstream occlusion (N=4 for each point)

DISCUSSION

Mechanisms of the myogenic response are still under investigation: the current study demonstrates a calcium-independent component to myogenic constriction. This is consistent with the report of Hill et al. [1], who found similar myogenic tone with a step increase in pressure compared to a ramp increase, with only the step increase giving a transient release of calcium. These results are also consistent with the findings of Civelek et al. [2], who found calcium-independent smooth muscle contraction induced by an increase in fluid shear stress. Flow across smooth muscle is relevent to the myogenic response in that fluid filtration from plasma to tissue must pass across smooth muscle in small pathways in which a significant amount of shear force is created [4]. Therefore, an interpretation of the findings of Civelek et al. is that a change in transvascular fluid filtration may initiate a myogenic response. In support of this hypothesis, our group has found the myogenic response to be dependent on fluid filtration [5], using a model similar to that described in the present study. We have found that the arteriolar myogenic response induced by downstream occlusion is reduced by ~50% when transvascular filtration is attenuated by the same percentage following injection of an osmotic solution of albumin and Ficoll [5]. Future studies may determine whether this filtration-dependent and calcium-independent mechanism relies on a rho-kinase pathway as found by Civelek et al. [2] for smooth muscle cell monolayers.

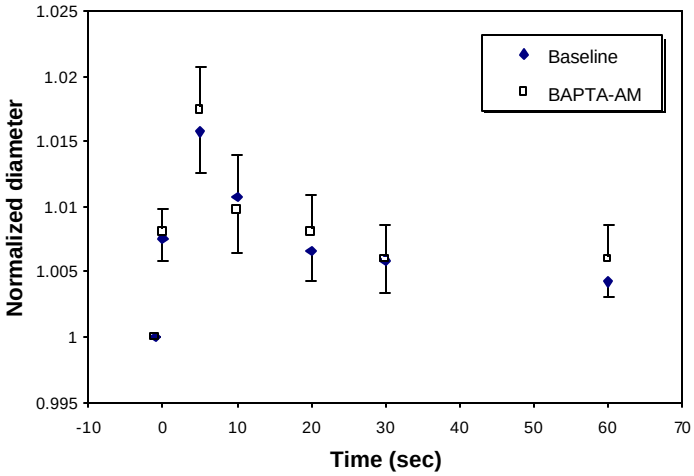

Figure 2. Arteriolar diameters normalized to values obtained prior to downstream occlusion (N=4)

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