Extracellular pH, Ca\(^{2+}\) Influx, and Response of Vascular Smooth Muscle Cells to 5-Hydroxytryptamine

Vitaly Nazarov, MD, PhD; Janette Aquino-DeJesus, MD; Michael Apkon, MD, PhD

**Background and Purpose**—Cerebral vascular smooth muscle cells (VSMCs) contract on extracellular pH (pH\(_e\)) increases and relax on pH\(_e\) decreases. These changes in tone are believed to result from changes in [Ca\(^{2+}\)], although the responsible mechanisms are not fully understood. VSMCs also contract in response to 5-hydroxytryptamine (5-HT), which increases [Ca\(^{2+}\)], via both Ca\(^{2+}\) release and influx. We hypothesized that examining effects of pH\(_e\), decreases on 5-HT–induced [Ca\(^{2+}\)], changes would allow us to identify mechanisms whereby pH\(_e\) influences tone. Accordingly, we compared [Ca\(^{2+}\)], increases in cerebral VSMCs, evoked by 5-HT, with increases evoked by increased pH\(_e\), and examined 5-HT–dependent [Ca\(^{2+}\)], increases at normal and decreased pH\(_e\).

**Methods**—We monitored [Ca\(^{2+}\)], using the Ca\(^{2+}\)-sensitive dye fura 2, in cultured rat cerebral VSMCs obtained by enzymatic digestion of middle cerebral arteries and their branches (passages 1 to 3) grown on glass coverslips and superfused with physiological saline.

**Results**—Increasing pH\(_e\) from 7.3 to 7.8 increased [Ca\(^{2+}\)], and these increases were prevented in Ca\(^{2+}\)-free solutions. Decreasing pH\(_e\) from 7.3 to 6.9 did not alter [Ca\(^{2+}\)], unless [Ca\(^{2+}\)]\(_i\) was first raised by treatment with 5-HT (10 \(\mu\)mol/L). 5-HT resulted in biphasic [Ca\(^{2+}\)], increases characterized by transient peaks blocked by the Ca\(^{2+}\)-ATPase inhibitor thapsigargin (10 nmol/L) and prolonged plateaux blocked by the Ca\(^{2+}\) channel blocker Ni\(^{2+}\) (1 mmol/L). Acidification did not alter the transient peaks but significantly reduced 5-HT–induced Ca\(^{2+}\) influx.

**Conclusions**—We conclude that increasing pH\(_e\) induces Ca\(^{2+}\) influx in rat cerebral VSMCs and decreasing pH\(_e\), inhibits 5-HT–stimulated Ca\(^{2+}\) entry but not intracellular Ca\(^{2+}\) release. (Stroke, 2000;31:2500-2507.)

**Key Words:** calcium ■ calcium channels ■ muscle, smooth ■ pH

Arterioles integrate a multitude of vasoactive inputs arising from neurohumoral signals, environmental cues such as pH, and mechanical factors such as flow and pressure. Each of these inputs may act directly on the vascular smooth muscle cell (VSMC) or may modulate the release of vasoactive substances from endothelial cells. Agents acting directly on the VSMC may alter tone by or may modulate the release of vasoactive substances from endothelial cells. Increasing pH\(_e\) from 7.3 to 7.8 increased [Ca\(^{2+}\)], while decreases on 5-HT–induced [Ca\(^{2+}\)], changes would allow us to identify mechanisms whereby pH\(_e\) influences tone. Accordingly, we compared [Ca\(^{2+}\)], increases in cerebral VSMCs, evoked by 5-HT, with increases evoked by increased pH\(_e\), and examined 5-HT–dependent [Ca\(^{2+}\)], increases at normal and decreased pH\(_e\).

**Materials and Methods**

**Preparation of Cerebral VSMC**

Cerebral VSMCs were isolated from adult rats by enzymatic dissociation of the intact MCA and its branches according to a previously published protocol. Briefly, adult male Sprague-Dawley rats (weight, 250 to 300 g) were anesthetized with methoxyflurane (Metafane, Pitman-Moore) and decapitated according to institutional guidelines. The skull was opened, the dura mater was stripped away.

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From the Departments of Pediatrics (V.N., J.A-D., M.A.) and Cellular and Molecular Physiology (M.A.), Yale University School of Medicine, New Haven, Conn.

Correspondence to Michael Apkon, MD, PhD, Department of Pediatrics, Yale University, PO Box 208064, New Haven, CT 06520-8064. E-mail michael.apkon@yale.edu

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and the brain was transferred to an ice-cold Pucks solution (GIBCO BRL) and then to the refrigerated stage (4°C) of a dissecting microscope. A section of cortex underlying the MCA was isolated, and the overlying pial membranes were gently removed, preserving the penetrating arterioles arising from the primary branches of the MCA. The MCAs and their first- and second-degree pial branches were dissected free of connective tissue and incubated in an enzyme solution containing collagenase, elastase, and deoxyribonuclease. After incubation, tissue culture medium (M-199; GIBCO BRL) supplemented with 10% fetal calf serum was added to terminate enzymatic digestion, and the cells were dissociated by gentle trituration. The cells were then centrifuged, resuspended, and plated onto 35-mm plastic tissue culture dishes or onto glass coverslips. The tissue culture medium (M-199) was supplemented with 10% fetal calf serum, insulin (5 µg/mL), selenium (5 ng/mL; ITS Premix, Collaborative Biomedical Products), penicillin (50 U/mL), and streptomycin (50 µg/mL; Pen-Strep, GIBCO BRL). After growing to confluence, the cells on plastic were harvested with trypsin (0.25%) and EDTA (1 mmol/L) in Ca-free PBS and passed onto glass coverslips or plastic culture dishes. The cells were studied in primary culture or at passages 1 to 3. Cells isolated and cultured in this manner maintain a contractile phenotype.5

Measurement of Intracellular Ca^{2+}

We measured [Ca^{2+}]_{i}, of cells grown on glass coverslips using ratiometric video fluorescence microscopy and the Ca^{2+}-sensitive dye fura 2.6 The coverslips were transferred from plastic tissue culture dishes into the bottom of an experimental chamber and placed against an oil-immersion objective on the inverted microscope. Cells were loaded with the cell-permeant acetoxymethyl ester fura 2-AM (Molecular Probes) at room temperature for 2 hours6 and then superfused with physiological saline solution for 30 minutes at 37°C to allow intracellular cleavage of the ester group before experiments were started. The fura 2-AM stock (1 mmol/L) was combined with Pluronic (20% wt/wt) in dimethyl sulfoxide) before dilution in tissue culture media to a final concentration of 10 µmol/L. Fura 2, 0.025% Pluronic. Fura 2 was excited at 340 and 380 nm, with emitted light intensity excited at each of the 2 wavelengths ($I_{340}/I_{380}$).

Our microscopy apparatus consisted of a Nikon Diaphot inverted microscope and a ×40 oil-immersion objective. The excitation source consisted of a light from a xenon arc lamp passing through a high-speed filter wheel (Ludl Electronics). The fluorescent images were detected by a variable gain intensified charge-coupled device camera (ICCD-350F, VideoScope), digitized by a video frame grabber (Occulus F64DSP, Coreco), and stored on the hard disk of a personal computer. Filter changing, specimen illumination, camera gain, image capture, and processing were all controlled by custom software developed with the use of Optimas (Optimas Corp).

Our software system incorporated camera gain control to ensure that the detected image intensities remained in the center of the camera’s linear range. Identical gains were used for the 2 images of each pair (1 data point), which allowed reliable intensity ratios to be calculated over a wide range of specimen intensities. The software also incorporated frame averaging, image thresholding, and dark current subtraction. For each area of interest, the pixel intensities of the paired images were averaged, and pixels with mean intensity values below a specified threshold did not contribute to the calculated average ratio. The average pixel intensities were calculated for the remaining pixels for each image, and the ratio of the average intensities was used to represent the value for the area of interest at that time point. Areas of interest were delineated by hand at the start of the experiment. We identified areas of interest encompassing the perinuclear regions of each cell in the microscope field. Typically, between 1 and 3 cells were examined in each experiment, and the results from each experiment were averaged together to establish the response for that particular experiment.

Given the difficulties in measuring reliable in vivo calibrations of fura 2, we inferred changes in [Ca^{2+}]_{i}, from changes in the ratio of emitted light intensity excited at each of the 2 wavelengths ($I_{340}/I_{380}$). We monitored $I_{340}/I_{380}$ at 60-second intervals during the 30-minute period of superfusion before starting the experiment and at 10-second intervals during the experiments. Percent changes in $I_{340}/I_{380}$ were used to compare the experimental results.

Measurement of Intracellular pH

Intracellular pH (pHi) was measured fluorometrically by a dual-excitation (440 versus 490 nm), single-emission (530 nm) ratiometric technique7 with the pH-sensitive fluorophore 2,7-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF; Molecular Probes Inc).9 Cells were loaded at 37°C for 30 minutes with BCECF (10 µmol/L), followed by a period of superfusion for 30 minutes with bath solution heated to 37°C. In each experiment, the fluorescence signals were calibrated using a variation of the high-[K^+] / nigericin technique,10 in which we calibrated the dye at the single pHi of 7.00. The ratio of light intensity excited at 490 nm to that excited at 440 nm measured at each data point is normalized to the ratio in each cell measured at pHi 7.00. pH is then calculated with the use of calibration curves spanning a pHi range from 6.5 to 7.8 generated in a population of cells. This approach allows a single point calibration and compensates for differences in the intensity of the excitation light across the microscope field and from experiment to experiment.

Solutions

The composition of the standard HEPES-buffered bathing solution was (in mmol/L) 145.8 Na, 3 K, 1 Ca, 1.2 Mg, 130 Cl, 1.2 SO_4, 2 PO_4, 32 HEPES, and 10.5 glucose. Ca^{2+}-free solutions were identical except that CaCl_2 was omitted. The pH of all solutions was measured and titrated to the required pH at 37°C with an Orion pH meter (model 811, Orion Research Inc). Solutions were delivered at 37°C by warming them in an in-line heater immediately before entering the experimental chamber.

Ketanserin (ICN Biomedicals) solutions were prepared daily by serially diluting a freshly prepared 20 mmol/L stock solution (in water) in the bathing solution to the final concentrations indicated. Nickel chloride was added to the bathing solution to the final concentration indicated. Thapsigargin (Sigma Chemical) solutions were prepared as a 5 mmol/L stock solution in water and diluted to a final concentration of 10 mmol/L in bath solution on the day of the experiments. 5-HT (Sigma Chemical) solutions were also prepared fresh daily.

Statistical Analysis

All results are expressed as mean±SD. Student’s paired t tests were used for statistical comparisons. The Bonferroni adjustment was applied for multiple comparisons. P<0.05 was considered statistically significant.

Results

Changes in pH_c, Cause Minimal Changes in pHi

In intact cerebral arterioles, changes in pH_c, caused much smaller changes in pHi (≈0.1 pH unit change in pHi per pH unit change in pH_c).11 To determine whether cultured cerebral VSMCs exhibit similar properties, we measured pHi, in cultured VSMCs during alkalination of the bath solution from pH 7.3 to 7.8. pHi, increased from 7.14±0.06 to 7.19±0.06 (n=8), although the mean pH_c values measured at pHi, 7.3 and 7.8 were not significantly different. The average change in pHi, was 0.05±0.006 pH units.

Changes in pH_o, Cause Changes in [Ca^{2+}]_i

To examine the direct effects of pH_o changes on [Ca^{2+}]_i, we measured [Ca^{2+}]_i, during increases and decreases in pH_o. In each experiment we compared the [Ca^{2+}]_i, increase of the VSMC during alkalination with the [Ca^{2+}]_i, increase during 5-HT application. As illustrated in Figure 1, increasing pHi, from pH 7.3 to 7.8 caused an increase in I_{340}/I_{380} that developed slowly, was maintained, and reversed when pHi,
was restored to 7.3. This cell also exhibited the typical response to 5-HT: a rapid rise to a transient peak, followed by a more slowly decaying component that produces an apparent plateau. Because the rates of this slower decay varied among experiments, we measured plateau values at specific times after the onset of 5-HT exposure. Figure 2 illustrates the mean (±SD) percent change in I_{340}/I_{380} for the response to alkanization as well as the peak and plateau (150 seconds after 5-HT exposure).

The alkanization-induced increase in [Ca^{2+}], required extracellular Ca^{2+}. As shown in Figure 3A, pH_{i} raised in a nominally Ca^{2+}-free bathing solution caused no increase in I_{340}/I_{380}. When Ca^{2+} was restored to the bathing solution while pH_{i} remained elevated, I_{340}/I_{380} promptly began to rise. The steady state percent change in I_{340}/I_{380} under each condition is shown in Figure 3B, along with the 5-HT–induced peak and plateau values.

Whereas increases in pH_{i} caused increases in [Ca^{2+}], decreases in pH_{i} infrequently caused decreases in [Ca^{2+}]. On average, the acidification-induced change in [Ca^{2+}] was no different than the small increase that was seen on average during sustained superfusion at constant pH_{i}. We reasoned that the failure to observe a decrease in [Ca^{2+}] with acidification could be caused by the following: (1) acidification relying on a mechanism independent of [Ca^{2+}], (2) the acidification-inhibitable mechanism being already quiescent under resting conditions, or (3) basal [Ca^{2+}] being sufficiently low that it is not possible to reliably measure a decrease with fura 2. We next examined the effects of pH_{i} decreases under conditions in which [Ca^{2+}] was already increased by application of 5-HT.

**Responses to 5-HT**

*Activation of 5-HT Receptor Increases [Ca^{2+}].* As described above, application of 5-HT (10 μmol/L) caused a rapid transient increase in [Ca^{2+}], followed by a slower
transient peak in the \([Ca^{2+}]\), waveform, although slower increases in \([Ca^{2+}]\), were observed in some cells. These increases were considerably smaller than those observed during the plateaus of the responses before thapsigargin exposure (Figure 4B). On average, 5-HT applied after exposure to thapsigargin did not raise \(I_{340}/I_{380}\) above the values measured immediately before the onset of that 5-HT application.

To test whether the plateau phases of the 5-HT–induced \([Ca^{2+}]\), responses reflect \(Ca^{2+}\) influx, we blocked \(Ca^{2+}\) entry using the \(Ca^{2+}\) channel inhibitor Ni\(^{2+}\). When Ni\(^{2+}\) (1 mmol/L) was added to the 5-HT–containing solution, the transient peak was still observed, but the plateau phase was inhibited. This did not simply reflect desensitization or tachyphylaxis to 5-HT because the effect was reversible: a third application of 5-HT after washing out Ni\(^{2+}\) from the bath resulted in a \([Ca^{2+}]\), increase similar to that observed during the first application (Figure 5A). Comparing the responses to the second 5-HT applications in the presence of Ni\(^{2+}\) with the response to 5-HT after washing out the Ni\(^{2+}\), we found that Ni\(^{2+}\) significantly inhibited only the plateau response (Figure 5B). Ni\(^{2+}\) alone had no effect on \(I_{340}/I_{380}\).

**Discussion**

**pH\(_{2}\) Dependence of \([Ca^{2+}]\)\(_{i}\)**

Vascular tone may be altered via changes in \([Ca^{2+}]\), or via changes in the sensitivity of the contractile/regulatory appa-
ratus to [Ca$^{2+}$]. We have found that for cerebral VSMCs, increased pH$_{o}$ increases [Ca$^{2+}$]. This pH$_{o}$ dependence of [Ca$^{2+}$] is also observed in mesenteric arterial smooth muscle cells, and the concurrent increase in tone in those cells reflects the increase in [Ca$^{2+}$] rather than from direct effects of the pH$_{o}$ increases. In cerebral VSMCs within intact arterioles, pH$_{i}$ changes relatively little when pH$_{o}$ is altered, and it is the pH$_{o}$ change rather than this pH$_{i}$ change that underlies the motor responses of cerebral arterioles and cultured cerebral VSMCs. The cultured VSMCs studied here behave similarly to those in arterioles in that they exhibit a nearly identical change in pH$_{i}$ with pH$_{o}$ changes (0.1 pH unit per 1 pH unit change in pH$_{o}$).

Whereas pH$_{o}$ increases led to [Ca$^{2+}$] increases, pH$_{o}$ decreases, on average, failed to alter [Ca$^{2+}$]. This is similar to the results of Dietrich and coworkers, who found that pH$_{o}$ decreases from 7.3 to 6.8 caused dilation of penetrating arterioles without significant decreases in [Ca$^{2+}$]. We may have failed to detect significant decreases in [Ca$^{2+}$] for several reasons. First, it is possible that the resting [Ca$^{2+}$] is already sufficiently low that I$_{340}$/I$_{380}$ is near R$_{min}$, the ratio observed in Ca$^{2+}$-free solutions. We think this unlikely given that the I$_{340}$/I$_{380}$ ratios we measured in vivo were higher than the ratios observed with our apparatus in vitro for Ca$^{2+}$-free solutions containing fura.

A second possibility is that, under resting conditions, the pH$_{o}$-responsive Ca$^{2+}$ influx process is inactive at rest, then one would expect that pH$_{o}$ decreases would not alter the resting tone in the cultured VSMCs. However, pH$_{o}$ decreases do relax cerebral VSMCs when they are grown on a flexible silicone substratum (dimethylpolysiloxane) on which they develop sponta-

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**Figure 5.** Requirement for Ca$^{2+}$ entry in the response to 5-HT. Time course of an experiment comparing the I$_{340}$/I$_{380}$ responses to 5-HT (10 µmol/L) before and after the cell was exposed to Ni$^{2+}$ (1 mmol/L). Note that the plateau of the 5-HT response is markedly diminished by the Ca$^{2+}$ channel blocker. The effect is reversible, as indicated by the response to a third 5-HT application. B, Ca$^{2+}$ increases (peak and plateau percent change in I$_{340}$/I$_{380}$) for the second and third 5-HT exposures are normalized to those recorded during the first exposure. Figure illustrates the mean and SD for percent changes in I$_{340}$/I$_{380}$ for the 5-HT--induced peak and plateau responses during the second and third 5-HT exposures for experiments such as that in A (n=3). The responses to the second and third exposures are compared with control for desensitization in the response to 5-HT. The p value indicates significance according to a paired Student’s t test with Bonferroni adjustment.

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**Figure 6.** Acidification reduces [Ca$^{2+}$] during the 5-HT--induced plateau (plat.). A, Time course of experiment demonstrating acidification to pH$_{o}$ 6.9 during exposure to 5-HT (10 µmol/L). I$_{340}$/I$_{380}$ was measured at the peak of the response (a), during the plateau immediately before reducing pH$_{o}$ (b), immediately before restoring pH$_{o}$ to 7.3 (c), and at the height of the 5-HT response after restoring pH$_{o}$. B, Summary (mean and SD) of percent change in I$_{340}$/I$_{380}$ (relative to the initial baseline value) for 7 experiments such as that in A. I$_{340}$/I$_{380}$ recorded at pH$_{o}$ 6.9 (c in A) is compared with the values measured before acidification (b) and after realkalinization (d). The p value indicates significance according to a paired Student’s t test with Bonferroni adjustment.
Alkalinization-induced increases in [Ca\(^{2+}\)]\(_{i}\) represent a balance point at which release from intracellular stores and influx from the extracellular space balance reuptake into the stores and extrusion from the cytoplasm, and each process is served by multiple mechanisms. Alkalinization-induced Ca\(^{2+}\) increases require extracellular Ca\(^{2+}\). This suggests that pH\(_{o}\) modulates the Ca\(^{2+}\) influx process rather than Ca\(^{2+}\) release from intracellular stores. This is corroborated by the effects of pH\(_{o}\), on 5-HT–induced [Ca\(^{2+}\)], increases. We cannot distinguish from these experiments whether pH\(_{o}\) affects Ca\(^{2+}\) influx directly via activating voltage-activated Ca\(^{2+}\) channels or indirectly by depolarizing the VSMC or stimulating some other Ca\(^{2+}\) influx process.

Mechanisms Underlying Alkalinization-Induced [Ca\(^{2+}\)]\(_{i}\) Increases

We chose to examine the response to 5-HT because this amine causes a biphasic increase in Ca\(^{2+}\) and because we believed it possible to separate 2 distinct processes leading to these Ca\(^{2+}\) increases. We applied relatively high doses of 5-HT to activate maximally the receptor-mediated transduction pathways. Our intention was to enable the dissection of the pathways leading to [Ca\(^{2+}\)]\(_{i}\) increases and to allow us to examine the effects of extracellular acidification on these pathways. We found that, in cerebral VSMCs, 5-HT induced a biphasic increase in [Ca\(^{2+}\)], that was blocked by the 5-HT\(_{2A}\) antagonist ketanserin. Inhibiting Ca\(^{2+}\) channels with the nonselective antagonist Ni\(^{2+}\) eliminates the plateau phase without altering the transient peak. Moreover, discharging the intracellular Ca\(^{2+}\) stores with thapsigargin, an endoplasmic reticulum Ca\(^{2+}\) pump inhibitor, eliminates the transient peak in [Ca\(^{2+}\)]. The failure to observe the peak responses after thapsigargin are not due to the fact that [Ca\(^{2+}\)], is elevated to a maximal level because thapsigargin does not cause elevations in [Ca\(^{2+}\)], itself as great as the peak responses to 5-HT observed before thapsigargin exposure. It is difficult to quantitatively compare the 5-HT–dependent increases in [Ca\(^{2+}\)], before and after thapsigargin treatment because [Ca\(^{2+}\)], levels often decay slowly after the thapsigargin is washed out of the bath solution. We conclude that 5-HT activates Ca\(^{2+}\) release followed by an increase in Ca\(^{2+}\) permeability of the cell.

Our differentiation of 2 modes for [Ca\(^{2+}\)], increases is consistent with the findings of other investigators. Transient 5-HT–induced, ketanserin-sensitive [Ca\(^{2+}\)], increases were observed in cultured rat cerebral VSMCs by Wang et al. These investigators also concluded that the transient increase resulted from Ca\(^{2+}\) release because Ca\(^{2+}\) channel antagonists such as Co\(^{2+}\), La\(^{3+}\), or nifedipine did not inhibit it. Interestingly, the [Ca\(^{2+}\)], increase observed by these investigators lasted only approximately 1 minute, and there was no sustained increase in [Ca\(^{2+}\)]. Ca\(^{2+}\) release has also been observed on 5-HT\(_{2}\) receptor activation in rat aortic VSMCs.
and VSMC cell lines,1,2 and Ca\(^{2+}\) release likely reflects generation of inositol(1,4,5)-trisphosphate.21

The later, sustained [Ca\(^{2+}\)]\(_i\) increase results from Ca\(^{2+}\) influx that, in cerebral VSMCs, is inhibited by Ni\(^{2+}\). The influx pathway is not known but could be either store-operated Ca\(^{2+}\) channels22 or voltage-operated Ca\(^{2+}\) channels. In rat aortic VSMCs, 5-HT\(_2\) receptor activation potentiates L-type Ca\(^{2+}\) currents via protein kinase C activation.23 5-HT also increases the open probability of the L-type Ca\(^{2+}\) current in rabbit cerebral arterioles.24 This is consistent with the finding that the Ca\(^{2+}\) channel antagonist verapamil reduces 5-HT–induced contraction of rat aortic strips and tail arteries.25 Others have found inconsistent antagonism of 5-HT\(_2\)-induced plateau [Ca\(^{2+}\)]\(_i\), increases with L-type Ca\(^{2+}\) channel antagonists and have suggested that 2 of these antagonists, verapamil and D600, interfere with binding of 5-HT to its receptor.3,26 Taken together, it seems likely that both store-operated and voltage-operated Ca\(^{2+}\) channels contribute to the plateau phase of the 5-HT\(_2\) response.

Acidification reduces the plateau Ca\(^{2+}\) increase but not the transient peak. The specificity of pH\(_i\), decreases for inhibiting the plateau, as opposed to the peak, suggests that the pH\(_i\) decrease does not interfere with 5-HT binding to its receptor or the generation of the intracellular second messengers leading to Ca\(^{2+}\) release. Rather, acidification specifically inhibits the Ca\(^{2+}\) influx process. This may reflect decreased L-type channel activity at low pH.27–29 It is also possible that low pH decreases Ca\(^{2+}\) influx through voltage-operated channels indirectly via change in membrane potential resulting from activation of K\(^+\) conductances.30 The [Ca\(^{2+}\)]\(_i\) decrease might also result from direct inhibition of the store-operated Ca\(^{2+}\) entry pathways, as observed in A7r5 rat aortic smooth muscle cells.31 The decrease in Ca\(^{2+}\) influx at acid pH may well underlie the decrease in maximal 5-HT–mediated contractions at acid pH in rabbit basilar artery.32

It is important to consider whether the responses to pH\(_i\) or 5-HT are general properties of cerebral arterioles or of all systemic VSMC. The cerebral VSMCs studied here derive from arterioles of varying size, from MCA to penetrating arterioles as small as 70 \(\mu\)m in diameter. These cells have a contractile response to 5-HT.5 Although the responses reported here were homogeneous, the response to locally applied 5-HT on pial vessels has been shown to vary with vessel size,33 with contraction of large vessels and dilation of small vessels. It is certainly possible that 5-HT causes different responses in vessels of different sizes or origins. This may relate to differences in the phenotypes of the vascular smooth muscle within the walls of the vessels or to differences in effects on smooth muscle compared with endothelium in different vessels. The importance of our results, however, is that pH\(_i\) modulates the Ca\(^{2+}\) influx pathway activated by 5-HT. This is particularly important since this Ca\(^{2+}\) influx pathway is likely activated by multiple vasoconstrictor substances. Moreover, this mechanism of [Ca\(^{2+}\)]\(_i\), regulation by pH is distinct from effects of pH\(_i\) on the intracellular release process.34

pH\(_i\) modulates vascular tone by altering Ca\(^{2+}\) influx, and the ability of pH\(_i\), to modulate [Ca\(^{2+}\)]\(_i\), depends on the resting activity of the various Ca\(^{2+}\) influx processes. Moreover, pH\(_i\), may modulate entry through store-operated Ca\(^{2+}\) channels as well as through voltage-operated Ca\(^{2+}\) channels. This underlies the importance of considering interaction between transduction processes when examining the influence of pH\(_i\), on vasomotor control.

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**Editorial Comment**

The strong sensitivity of cerebral vascular tone to changes in extracellular pH is one of the features that differentiates cerebrovascular blood flow regulation from other vascular networks. As such, understanding the cellular mechanisms by which metabolic need of the cerebral tissue, as reflected by tissue pH, causes alkalotic smooth muscle constriction or acidotic dilation is an important issue. To this end, Nazarov et al observed the intracellular calcium activity in cultured cerebral macrovessels and microvessels, alkalosis-induced constriction and may be a safeguard mechanism to allow for higher blood flow in situation of local metabolic need.

Several questions regarding the mechanism of pH-induced vasomotor responses still remain. A previous study of this group\(^1\) showed that cerebral smooth muscle cells in culture relax to acidic pH. Since the present study found no decrease in calcium activity, another calcium-independent mechanism needs to be postulated to explain the acidic relaxation.

Further, the nature of the observed alkalotic calcium channel activation is still unexplained. As stated by the authors, this study does not discern whether alkalosis directly opens calcium channels and, if so, which calcium channel type may be involved. Alternatively, because increased pH depolarizes cerebral macrovessels and microvessels,\(^2\).\(^3\) alkalosis-induced calcium influx could be secondary to membrane depolarization. As such, the primary effect of increased extracellular pH on the smooth muscle needs to be elucidated further.

**Hans H. Dietrich, PhD, Guest Editor**
**Department of Neurosurgery**
**Washington University School of Medicine**
**St Louis, Missouri**

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