mice carrying H-2^b allogeneic or H-2^k syngeneic pregnancies (day 10). Leukocytes were identified by a CD45-specific mAb (30F11.1, Pharmingen); T cells were identified by an unconjugated rat clonotype-specific mAb (B20.2.2) and by commercial (Gibco) biotinylated mAbs specific for CD3 (29B) and TCR$\alpha\beta$ (H57-597). Unconjugated primary antibodies were detected with a biotinylated sheep antibody to rat serum. Biotinylated antibodies were revealed by a streptavidin-biotinylated peroxidase complex. The numbers of CD45-positive cells were comparable in midpregnant allogeneic and syngeneic uteri. Maternal T cells were numerous in the decidua but were absent from the placenta and fetus.

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20. H-2^d P815-K^b mastocytoma cells implanted subcutaneously are rejected by H-2$^{d\times k}$ Des-TCR mice but are accepted by Des-TCR mice tolerant to K^b expressed under the Keratin-IV promoter (2.4Ker.K^b × Des-TCR) (*24*). P815-K^b elimination is mediated by Des-TCR T cells. In vivo depletion of Des-TCR T cells by Désiré-1 mAb, but not of natural killer cells by NK1.1 mAb, blocked tumor rejection (A. Limmer, G. J. Hämmerling, B. Arnold, unpublished data). Rejection was verified for more than 3 months. When the tumor diameter exceeded 2 cm after increasing for more than two consecutive measurements, tumor-bearing mice were killed, and K^b expression was tested on P815-K^b cells ex vivo.
21. Among all peptides extracted from K^b molecules [C57BL/6 spleen, EL-4, or RMA (H-2^b) tumor cells] and separated by high-performance liquid chromatography, only one and the same peptide fraction sensitized RMA-S cells for recognition by a Des-TCR–positive clone. Therefore, the Des-TCR–positive clone recognizes only one (or few) peptides in the context of K^b. The peptide sequence is currently being investigated (A. Guimezanés and A.-M. Schmitt-Verhulst, personal communication).
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Relaxation of Arterial Smooth Muscle by Calcium Sparks

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Local increases in intracellular calcium ion concentration ($[Ca^{2+}]_i$) resulting from activation of the ryanodine-sensitive calcium-release channel in the sarcoplasmic reticulum (SR) of smooth muscle cause arterial dilation. Ryanodine-sensitive, spontaneous local increases in $[Ca^{2+}]_i$ (Ca^{2+} sparks) from the SR were observed just under the surface membrane of single smooth muscle cells from myogenic cerebral arteries. Ryanodine and thapsigargin inhibited Ca^{2+} sparks and Ca^{2+}-dependent potassium (K_{Ca}) currents, suggesting that Ca^{2+} sparks activate K_{Ca} channels. Furthermore, K_{Ca} channels activated by Ca^{2+} sparks appeared to hyperpolarize and dilate pressurized myogenic arteries because ryanodine and thapsigargin depolarized and constricted these arteries to an extent similar to that produced by blockers of K_{Ca} channels . Ca^{2+} sparks indirectly cause vasodilation through activation of K_{Ca} channels, but have little direct effect on spatially averaged $[Ca^{2+}]_i$, which regulates contraction.

Myogenic arteries control blood flow in the brain and respond to changes in intravascular pressure. Increased intravascular pressure causes a graded membrane potential depolarization of smooth muscle cells and arterial constriction (myogenic tone) (*1–3*). Small, pressurized cerebral arteries dilate when the membrane potential of the smooth muscle cells is made more negative over the physiological range of membrane potentials (−60 to −30 mV), because steady Ca^{2+}-influx through dihydropyridine-sensitive, voltage-dependent Ca^{2+} channels declines (*2–4*). Ca^{2+} entry at physiological membrane potentials affects spatially averaged $[Ca^{2+}]_i$ in arterial smooth muscle (*3*, *4*). Although ryanodine-sensitive Ca^{2+}-release channels directly contribute to the global $[Ca^{2+}]_i$ transient and contraction in cardiac muscle (*5*), their functional role in smooth muscle has not been established (*4*, *6*, *7*). We monitored elementary ryanodine-sensitive Ca^{2+}-release events (Ca^{2+} sparks) from smooth muscle SR by measuring rapid local changes in $[Ca^{2+}]_i$ in smooth muscle cells isolated from resistance-sized cerebral arteries. We provide evidence that ryanodine-sensitive Ca^{2+}-release channels in smooth muscle SR, unlike their counterparts in cardiac and skeletal muscle, have a central role in limiting muscle contraction by activating K_{Ca} channels.

Single smooth muscle cells were isolated enzymatically from myogenic cerebral (100- to 150-μm in diameter posterior and middle cerebral) arteries from rat (*8*). We used a laser scanning confocal microscope and the fluorescent Ca^{2+} indicator fluo-3 (*9*) to detect Ca^{2+} sparks in single cells bathed in physiological salt solution (Figs. 1 and 2). The mean rise-time and half-time of decay of Ca^{2+} sparks were 20.2 ± 2.3 ms and 48.0 ± 2.6 ms (n = 11), respectively (Fig. 1). The mean peak $[Ca^{2+}]_i$ during the Ca^{2+} spark was 303 ± 27 nM (assuming 100 nM resting Ca^{2+}) (Fig. 1) (*10*). The mean spread of the spark at the peak was 2.38 ± 0.14 μm (n = 11) (Fig. 1) (*10*), corresponding to 0.8% of the surface area of the cell membrane (*11*).

Ryanodine, which inhibits SR Ca^{2+}-release channels at micromolar concentrations (*5*, *6*), blocked Ca^{2+} sparks in smooth muscle cells (Fig. 2B). Ca^{2+} sparks were not observed in cells exposed to 10 μM ryanodine and the Ca^{2+} channel agonist Bay K 8644, whereas 88% of cells treated with Bay K 8644 alone had Ca^{2+} sparks. Ca^{2+} sparks were not observed in cells exposed to thapsigargin (1 μM), which inhibits Ca^{2+} uptake into the SR by the Ca^{2+}-ATPase (*12*). Application of cadmium (200 μM), which immediately blocks voltage-dependent Ca^{2+} channels, did not immediately block Ca^{2+} sparks in our cells (n = 7), a finding similar to that observed in quiescent heart muscle cells (*5*). However, prolonged exposure to Bay K 8644 increased Ca^{2+}-spark occurrence (Fig. 2B). The majority of Ca^{2+} sparks (59%) arose close to the sarcolemmal surfaces (within 1 μm) of the smooth muscle cells (Fig. 2C). The Ca^{2+} sparks that were detected in the middle of the line-scan may still have arisen at the sarcolemmal surface because smooth muscle cells have infoldings of the surface membranes (caveolae). These results suggest that most Ca^{2+} sparks in smooth muscle cells from resistance-sized cerebral arteries result from the opening of ryanodine-sensitive Ca^{2+}-release channels in SR just under the cell membrane.

The proximity of the Ca^{2+} sparks to the cell surface raises the possibility that the Ca^{2+} spark serves as an intracellular signal to the sarcolemmal membrane. Ca^{2+}-activated K^+ (K_{Ca}) channels that exist in this membrane (*2*, *3*) should be activated by the

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local increase in $[Ca^{2+}]_i$ produced by the Ca^{2+} sparks. To examine this possibility, we used the perforated-patch method to measure membrane currents in intact single cells (*13*). The membrane potential was held at −40 or −30 mV, similar to that of smooth muscle cells in the intact pressurized artery and in freshly dissociated smooth muscle cells (*1–3*). Outward current transients were observed (Figs. 1 to 3) with a time course similar to that of the Ca^{2+} spark and were completely inhibited by the application of ryanodine (10 μM) (n = 6) (Fig. 3A) or thapsigargin (100 nM) (n = 3) (Fig. 3B), suggesting that they were activated by the local $[Ca^{2+}]_i$ increase produced by individual Ca^{2+} sparks. Tetraethylammonium ions (TEA^+) (1 mM) (Fig. 3C) and

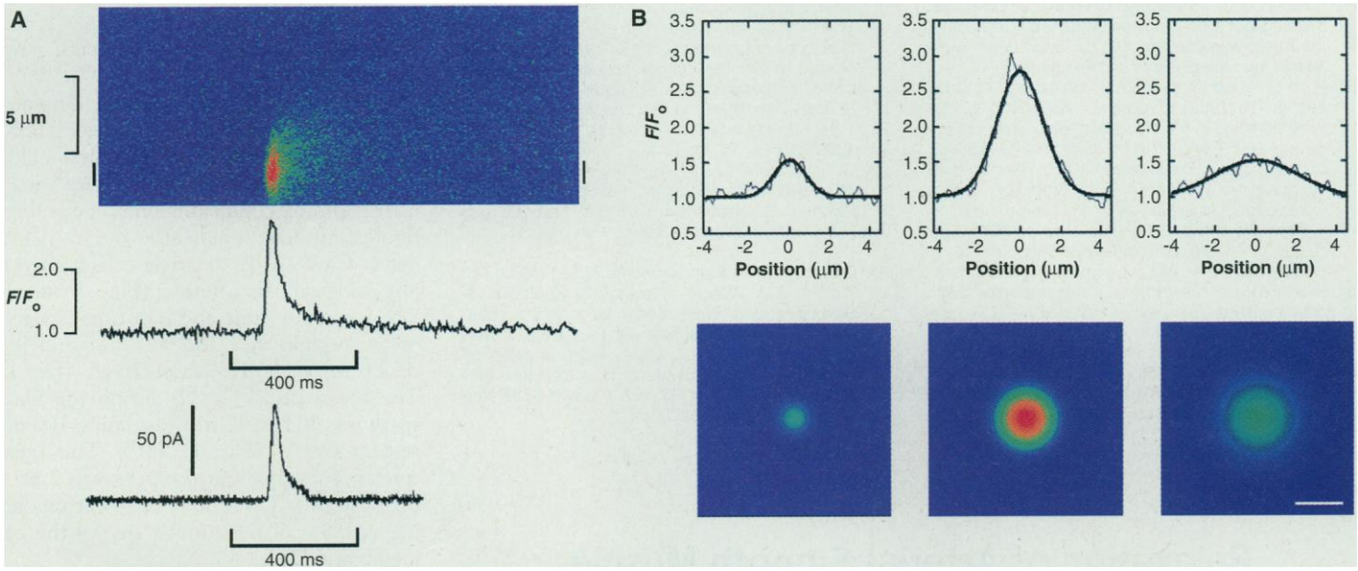
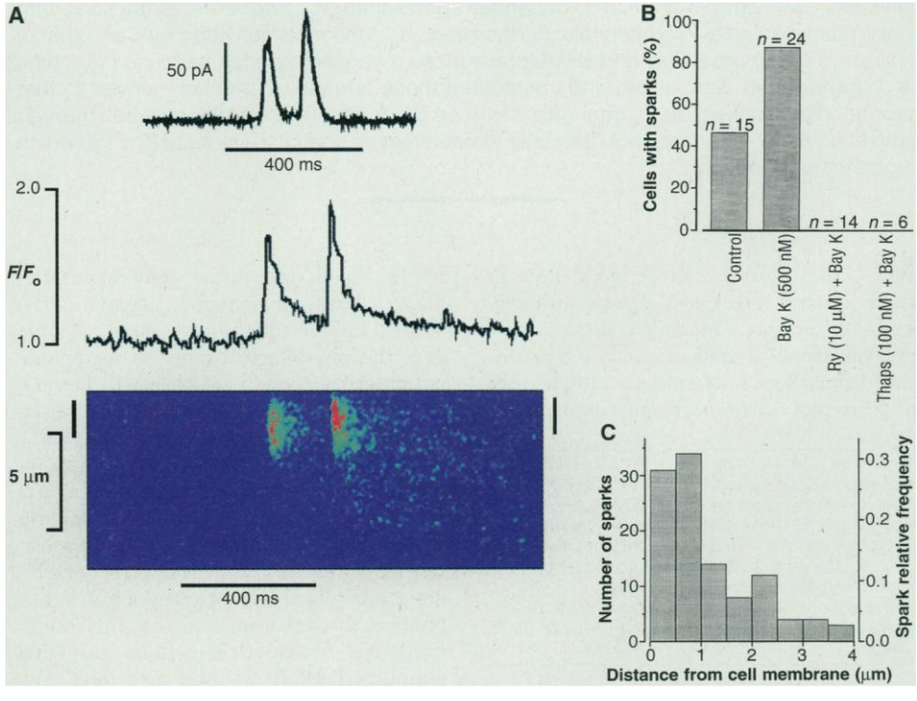


Fig. 1. Spatial-temporal characteristics of a Ca^{2+} spark in a smooth muscle cell from a rat posterior cerebral artery. (**A**) Confocal line-scan image of a fluo-3–loaded cerebral artery smooth muscle cell, with the time course indicated below. The fluorescence time course of the Ca^{2+}-spark was averaged over the region indicated by the bar. Bottom trace shows an example of a spontaneous transient outward current (STOC) from a different cell (at −40 mV) (Fig. 3). Each line-scan image is a plot of fluorescence along a scanned line (that is, position) on the ordinate versus time (on the abscissa) (*5*). The line-scan image duration was 1536 ms, and each line was 6 ms. (**B**) Spatial-temporal characteristics of the spark shown in (A). (**Upper panels**) Spatial distribution of Ca^{2+} fit by a Gaussian (solid line), relative to the initiation point of the spark. Shown are the spatial distributions at the first indication of an increase in Ca^{2+} (left), at the peak Ca^{2+} (middle), and 66 ms later (right). The full width at half-maximal $[Ca^{2+}]$ at the three time points of the spark life cycle were 1.84, 2.65, and 4.99 μm. (**Lower panels**) Corresponding estimated spread of a spark by rotation of the fit Gaussian 360°. Bar, 3 μm. Single smooth muscle cells were isolated enzymatically from 100-μm-diameter myogenic posterior cerebral arteries from rat as described (*8*). Bath solution: 6 mM KCl, 134 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, and 10 mM glucose (pH 7.4) at room temperature.

Fig. 2. Calcium sparks in single smooth muscle cells isolated from myogenic cerebral arteries: Inhibition of Ca^{2+} sparks by ryanodine and thapsigargin and localization next to the surface membrane. (**A**) Line-scan image illustrating two sparks at the edge, with the time course of the two sparks and an example of two STOCs (from a different cell, at −40 mV) above the image. Bar on line-scan image, 1.5 μm. (**B**) Percentage of cells exhibiting one or more sparks during 30-s scanning with Bay K 8644 (500 nM), thapsigargin (Thaps; 1 μM) + Bay K, and ryanodine (Ry; 10 μM) + Bay K. The cells were incubated with each drug for at least 10 min before being examined for sparks. Each cell was scanned for a average total time of 30 s. Longer scanning resulted in bleaching of the dye. Assuming that 1% of the cell volume was scanned for 30 s and a spark frequency of 1/s (on the basis of STOC measurements), a spark should have been observed in about 30% of the control cells. The total number of cells examined under each condition is indicated above each bar. (**C**) Frequency of sparks as a function of distance from edge of the cell. The edges of the cells correspond to the upper and lower edges of the line-scan image.



iberiotoxin (100 nM) (Fig. 3D) completely blocked the currents, confirming the identification of the current source as K_{Ca} channels (*2*, *3*, *14*). Ryanodine-sensitive, spontaneous transient outward currents (STOCs) through K_{Ca} channels have been observed in a number of other types of smooth muscle (*15*, *16*). At −40 mV, the STOCs we measured had a mean amplitude of 21.5 ± 6.4 pA (n = 7), a mean duration of 64.9 ± 7.9 ms, a mean rise time of 17.1 ± 1.6 ms, and a mean frequency of 1.28 ± 0.42 Hz (n = 6) in intact quiescent cells. Thus, the kinetics of the STOCs were similar to those of the Ca^{2+} sparks. Ryanodine appeared not to inhibit STOCs by blocking K_{Ca} channels, because neither ryanodine (10 μM) nor thapsigargin (1 μM) decreased the open-state probability or unitary current of single K_{Ca} channels in excised patches (Fig. 3 legend). The minimum number of K_{Ca} channels activated during a STOC can be estimated by dividing the mean STOC amplitude (21.5 pA) at −40 mV by the mean single-channel current (1.6 ± 0.1 pA; n = 3) (*17*). The results support the idea that a single ryanodine-sensitive Ca^{2+} spark from the SR activates at least 13 K_{Ca} channels to produce a single STOC.

It would thus be expected that Ca^{2+} sparks, by activating K_{Ca} channels, would hyperpolarize the membrane potential of myogenic cerebral arteries and so result in vasodilation (*2*, *3*, *16*, *18*). The myogenic response to an increase in intravascular

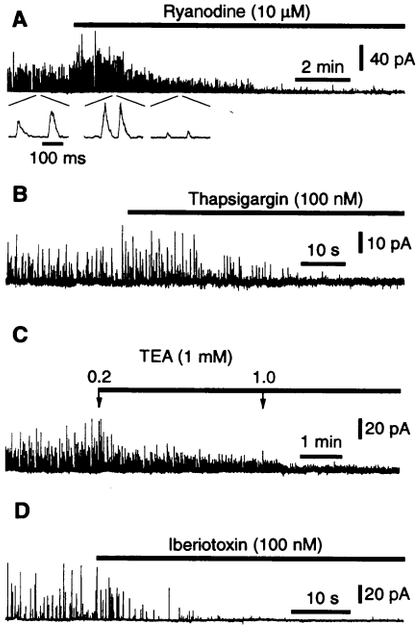


Fig. 3. Inhibition of spontaneous transient outward currents by ryanodine, thapsigargin, TEA^+, and iberiotoxin. (**A**) Ryanodine (10 μM) block of STOCs. (Below: expanded time course before, shortly after, and during addition of ryanodine before cessation of STOCs.) Ryanodine has no direct effect on K_{Ca} channels [NP_o (ryanodine)/NP_o (control) = 0.97 ± 0.02 (SE; n = 3)], and unitary currents at 0 mV were 5.94 ± 0.3 pA (control) and 5.71 ± 0.34 pA (ryanodine). (**B**) Thapsigargin (100 nM) block of STOCS. Thapsigargin has no direct effect on K_{Ca} channels [NP_o (thapsigargin)/NP_o (control) = 0.928 ± 0.18 (SE; n = 4)], and unitary currents at 0 mV were 6.1 ± 0.2 pA (control) and 6.1 ± 0.3 pA (thapsigargin). (**C**) Tetraethylammonium (TEA^+) block of STOCs. TEA^+ at 0.2 mM reduced STOC amplitudes by 50%, which is the same as the concentration of TEA^+ required to inhibit single K_{Ca} channels by 50% (*14*). (**D**) Iberiotoxin (100 nM) block of STOCs. Whole-cell currents in single smooth muscle cells isolated from myogenic cerebral arteries of rat were measured with the perforated-patch configuration of the whole-cell recording technique. Bath solution: 6 mM KCl, 134 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, 10 mM glucose (pH 7.4); pipette solution: 30 mM KCl, 110 mM potassium aspartate, 10 mM NaCl, 1 mM $MgCl_2$, 50 μM EGTA, amphotericin (200 mg/ml) (pH 7.2) at room temperature.

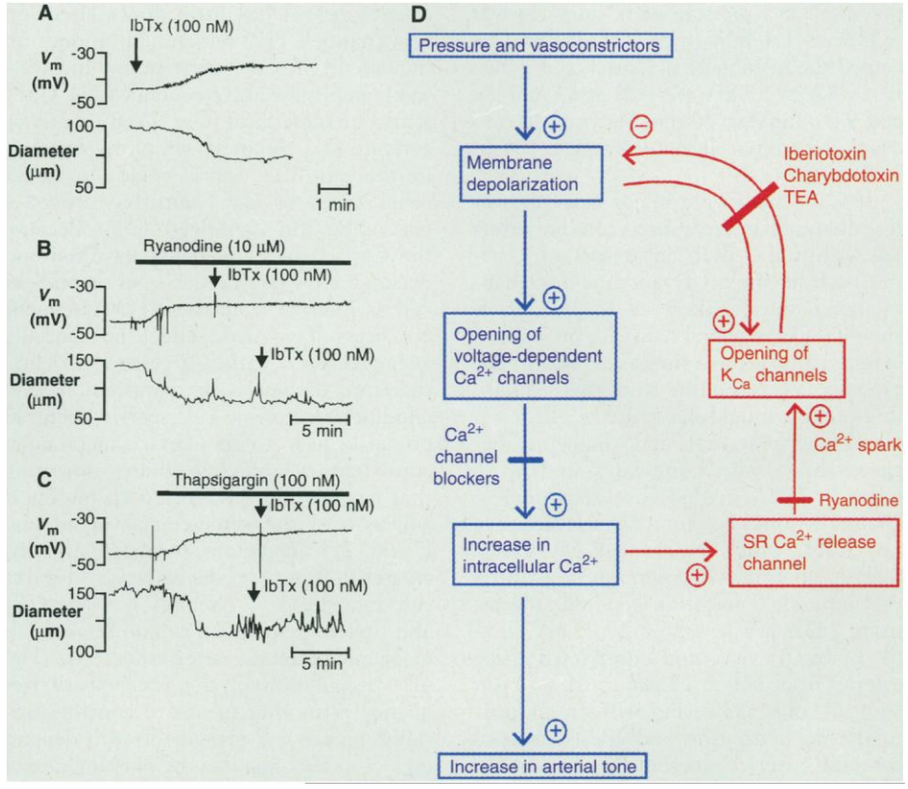


Fig. 4. Depolarization and constriction of rat myogenic cerebral arteries with tone by inhibitors of K_{Ca} channels (iberiotoxin) (**A**) and Ca^{2+} sparks [ryanodine (**B**) and thapsigargin (**C**)]. Cerebral arterial pressure was 60 mm Hg in all experiments. Iberiotoxin alone depolarizes and constricts. Ryanodine (10 μM) depolarizes and constricts, and addition of iberiotoxin (arrow) had no effect on membrane potential and diameter in the presence of ryanodine (n = 5). Thapsigargin (100 nM) depolarizes and constricts, but iberiotoxin (100 nM) was without effect in the presence of thapsigargin. The mean diameter of the arteries at 60 mm Hg dilated with 100 nM nisoldipine was 228 ± 43 μm (n = 17). The diameter of the pressurized arteries was measured by a video image analyzer (Living Systems Instruments, Burlington, Vermont), and the membrane potential was measured with conventional microelectrodes as described (*2*). Bath solution: 119 mM NaCl, 4.7 mM KCl, 24 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.023 mM EDTA, 11 mM glucose (pH 7.4) with continuous bubbling with 95% O_2–5% CO_2 at 37°C. The diameter and membrane potential measurements were made in different arteries. (**D**) Proposed model for the roles of K_{Ca} channels and ryanodine-sensitive Ca^{2+}-release channels in the SR (Ca^{2+} sparks) in the regulation of arterial tone. In this model, Ca^{2+} sparks have a key role in the negative-feedback regulation of arterial tone through activation of K_{Ca} channels (right limb of diagram in red). Intravascular pressure causes a maintained membrane potential depolarization and constriction (myogenic tone) of small myogenic cerebral arteries (*1*–*3*). Myogenic tone is blocked by the removal of external Ca^{2+}, voltage-dependent Ca^{2+}channel blockers, or membrane hyperpolarization (*1*–*3*). Activation of Ca^{2+} channels increases Ca^{2+} entry and spatially averaged Ca^{2+} in the smooth muscle (*3*, *22*, *29*). This small increase in spatially averaged Ca^{2+} produces a steep increase of force (*22*) (left limb of diagram in blue), which would result in an increase of SR Ca^{2+}content. This should increase Ca^{2+} spark frequency and amplitude and thereby activate K_{Ca} channels (*23*). Other important elements in the control of arterial tone not shown include inositol trisphosphate (IP_3)–induced Ca^{2+} release, Ca^{2+} extrusion and uptake mechanisms, and mechanisms to change Ca^{2+} sensitivity (*7*). This model also suggests a mechanism by which vasodilators and vasoconstrictors could regulate arterial tone through modulation of Ca^{2+} spark frequency and amplitude.

pressure from 10 mm Hg to 60 to 80 mm Hg involves the depolarization of the smooth muscle membrane potential from about −60 to about −40 mV, the opening of voltage-dependent Ca^{2+} channels, increased $[Ca^{2+}]_i$, and the consequent constriction of the vessel by about 30 to 40% (*1–3, 7, 19*). Blockers of K_{Ca} channels (such as charybdotoxin and iberiotoxin) depolarize the membrane potential of smooth muscle cells by 7 to 9 mV and constrict myogenic pressurized cerebral arteries by about 40% (*2*) (Fig. 4A). The mean membrane potential at a pressure of 60 mm Hg was −43.9 ± 1.4 mV (n = 12). Iberiotoxin caused the membrane potential to depolarize by 8.6 ± 2.3 mV (n = 3) at 60 mm Hg and 9 ± 2 mV at 80 mm Hg (n = 6) and decreased arterial diameter by 41 ± 9% (n = 6) (*2*).

If Ca^{2+} sparks are major activators of K_{Ca} channels in pressurized cerebral arteries with tone, then inhibitors of Ca^{2+} release from the SR (ryanodine and thapsigargin) and blockers of K_{Ca} channels should depolarize and constrict pressurized cerebral arteries to a similar extent. In the presence of ryanodine and thapsigargin, iberiotoxin should have little effect on membrane potential and diameter, because the localized increases in $[Ca^{2+}]_i$ (that is, Ca^{2+} sparks) that activate K_{Ca} channels would be eliminated. Ryanodine (10 μM), which blocks the SR Ca^{2+}-release channel, depolarized pressurized (60 mm Hg) posterior cerebral arteries from −43.9 mV to −36.6 ± 0.8 mV (n = 6), or by 7.3 mV, and constricted these arteries from 121 ± 12 μm to 81 ± 7 μm (n = 6), or by 33% (Fig. 4B). In contrast to the depolarization and constriction of cerebral arteries caused by iberiotoxin alone (*2*), this K_{Ca} channel blocker had no effect on membrane potential or diameter after application of ryanodine (n = 5) (Fig. 4B) (*20*). Similarly, after thapsigargin had been applied [to block SR Ca-ATPase (*12*)], the addition of iberiotoxin did not affect membrane potential or diameter (Fig. 4C). Thapsigargin (100 nM) alone depolarized and constricted the pressurized (60 mm Hg) cerebral arteries by 6.9 ± 1.4 mV (n = 4) and from 122 ± 13 mV (n = 5) to 96 ± 12 mV (n = 4), respectively (n = 3) (Fig. 4C). Furthermore, the effects of thapsigargin were tested in arteries denuded of the endothelium to eliminate any possible complications from alterations in nitric oxide release (*21*). Thapsigargin also constricted these pressurized cerebral arteries from 95 ± 14 μm to 62 ± 10 μm (n = 3), or by 35%. Another inhibitor of the SR Ca-ATPase, cyclopiazonic acid (10 μM), also constricted pressurized cerebral arteries (from 123 ± 10 μm to 77 ± 3 μm) (n = 3). These results suggest that blockers of Ca^{2+} sparks depolarize and constrict myogenic cerebral arteries by inhibiting K_{Ca} channels.

An increase in global $[Ca^{2+}]_i$ caused by raised intravascular pressure is due to Ca^{2+} influx through voltage-sensitive Ca^{2+} channels and results in an increase in force generation (*7, 22*) by means of myosin light chain kinase activated by Ca^{2+}-calmodulin (Fig. 4D). This modest increase in $[Ca^{2+}]_i$ (compared with the large local increase in $[Ca^{2+}]$ produced by a Ca^{2+} spark) has little direct effect on K_{Ca} channels (*17*) but should produce an increase in SR Ca^{2+} content and in Ca^{2+}-spark amplitude and frequency (*23*). Ca^{2+} sparks increase local $[Ca^{2+}]$ sufficiently to activate K_{Ca} channels, which hyperpolarize the cell. Ca^{2+} sparks could also affect other types of Ca^{2+}-sensitive processes (including ion channels) (*24*). Because the Ca^{2+} sparks are highly localized and occur at a low rate (about 1 per second per cell as inferred from the STOC frequency), they have little effect on spatially averaged Ca^{2+} within a cell (*4, 25*) and therefore do not cause contraction. Ryanodine-sensitive Ca^{2+} sparks occurred primarily next to the surface membrane, consistent with previous studies indicating that much of the SR in smooth muscle is adjacent to the sarcolemmal membrane (*7, 26, 27*). Therefore, localized large increases in $[Ca^{2+}]$ produced by Ca^{2+} sparks can increase K_{Ca} channel activity (*17*) and thereby hyperpolarize and relax small myogenic cerebral arteries (*2, 3*) (Fig. 4D). Regulation of the Ca^{2+} spark frequency is another means to control arterial diameter and presumably will depend on factors that regulate the opening rate of the ryanodine receptors (SR Ca^{2+}-release channels), such as the phosphorylation state and $[Mg^{2+}]$.

Blocking Ca^{2+} sparks or K_{Ca} channels in pressurized, myogenic arteries would then cause membrane depolarization and vasoconstriction (*16, 27*) (Fig. 4). The lack of effect of iberiotoxin in the presence of ryanodine or thapsigargin (that is, when Ca^{2+} sparks are blocked) (Fig. 4, B and C) suggests that average cytoplasmic Ca^{2+} in the absence of Ca^{2+} sparks does not cause sufficient activation of K_{Ca} channels to regulate smooth muscle membrane potential (Fig. 4D). However, inhibitors of voltage-dependent Ca^{2+} channels decrease average $[Ca^{2+}]_i$ and relax myogenic cerebral and skeletal muscle arteries in the presence of ryanodine (*28*). These findings indicate that Ca^{2+} entry can influence spatially averaged $[Ca^{2+}]_i$, which in turn regulates contraction (Fig. 4D). In conclusion, we propose that the ryanodine-sensitive Ca^{2+}-release channel has a key function in controlling the diameter of small myogenic arteries through the regulation of K_{Ca} channels. These results suggest a mechanism for control of vasodilation and constriction through modulation of the amplitude and frequency of Ca^{2+} sparks.

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9. A Bio-Rad MRC600 (Cambridge, MA) confocal scanning head connected to a Nikon Diaphot microscope was used to image the cells with COMOS and SOM software (Bio-Rad). Images were acquired in line-scan mode of the confocal microscope; this mode repeatedly scans a single line through the cell every 6 ms. The line-scan traverses the entire width of a cell. The line-scan results are displayed vertically and each line is added to the right of the preceding line to form the line-scan image. In these images, time is in the horizontal direction (left to right) and position along the scan line is given by the vertical displacement. The resolution of the microscope is approximately 0.4 μm by 0.4 μm (x and y) by 0.8 (z) μm with a Zeiss Neofluor 63 1.25 NA objective, as measured with 0.09-μm fluorescent beads (Molecular Probes, Eugene, OR). IDL software (Boulder, CO) was used for data analysis. The fluorescence record was normalized by dividing the fluorescence traces by the average fluorescence during the prestimulus period. Calibration of the fluo-3 signal was done as described (*5*). The cells were loaded with fluo-3 by a 10-min incubation with 5 μM fluo-3 AM (Molecular Probes) followed by a 30-min wash. The site of origin of a Ca^{2+} spark was determined as the center of the spark at the time of its initiation.
10. Although the peak $[Ca^{2+}]_i$ of a spark and the spatial profile of the Ca^{2+} sparks are similar in cerebral artery smooth muscle cells and cardiac myocytes (*5*), the kinetics of Ca^{2+} sparks in smooth muscle cells are slower. The slower kinetics could arise from different ryanodine-sensitive Ca^{2+}-release channel kinetics or altered properties of the SR Ca-ATPases and Ca^{2+} buffers. The increase of $[Ca^{2+}]_i$ occurring during a Ca^{2+} spark is consistent with either the opening of one or the coordinated opening of a small number of colocalized ryanodine-sensitive Ca^{2+}-release channels (*5*).
11. Single smooth muscle cells from posterior cerebral

arteries of rat have a mean membrane capacitance of 10.8 ± 0.4 pF (n = 47) (*8*), corresponding to a membrane surface area of about 1080 μm^2. The fraction of the surface area experiencing a spark was estimated as the surface area of a hemisphere (about 9 μm^2) with a radius (1.19 μm) of the mean spread of a spark divided by the cell surface area (1080 μm^2). The volume of a single cell was estimated to be about 1 pl.

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17. Local $[Ca^{2+}]_i$ increased as much as fivefold (mean, threefold) during a Ca^{2+} spark. A fivefold increase in $[Ca^{2+}]_i$ has been shown to shift the activation curve of single K_{Ca} channels by about 80 mV [C. L. Kapicka *et al.*, *Am. J. Physiol.* **266**, C601 (1994)]. This would increase channel open-state probability (P_o) by as much as 1000-fold. In smooth muscle cells from mesenteric arteries, increasing $[Ca^{2+}]_i$ from 200 nM to 1 μM increased the P_o of K_{Ca} channels in inside-out excised patches from about 0.003 (estimated) to about 0.5 at −40 mV [C. D. Benham, T. B. Bolton, R. J. Lang, T. Takewaki, *J. Physiol.* **371**, 45 (1986)]. Assuming that a cerebral artery myocyte has about 10,000 uniformly distributed K_{Ca} channels (*3*), the mean spread of a Ca^{2+} spark could affect about 70 channels. The average STOC corresponded to 13 open K_{Ca} channels, or an average local P_o of 0.18 during a spark.
18. Very few K^+ channels need to open to cause a membrane hyperpolarization of arterial smooth muscle (*3*), because arterial smooth muscle cells have high input resistances (10 gigohms) at physiological membrane potentials in the absence of STOCs. Therefore, the membrane potential would hyperpolarize towards the K^+ equilibrium potential (−85 mV) during an average STOC (*16*). STOCs in smooth muscle cells in the arterial wall would sum to cause a graded membrane potential hyperpolarization. The average membrane potential would reflect the contribution of K_{Ca} conductance caused by Ca^{2+} sparks and other conductances.
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20. In the presence of ryanodine or thapsigargin, the inability of iberiotoxin to depolarize and constrict the arteries further appeared not to be limited by some intrinsic property of the vessels, because other agents, such as 4-aminopyridine or high concentrations of K^+, caused further membrane potential depolarization (to about −22 mV) and constriction of the arteries (to <50 μm) (*2*).
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24. $[Ca^{2+}]_i$ has been shown in some types of smooth muscle to inhibit voltage-dependent K^+ channels [C. H. Gelband, T. Ishikawa, J. M. Post, K. D. Keef, J. R. Hume, *Circ. Res.* **73**, 24 (1993)] and to activate Cl^- channels [R. C. Hogg, Q. Wang, R. M. Helliwell, W. A. Lange, *Pflügers Arch.* **425**, 233 (1993)]. In both cases, Ca^{2+} sparks would be expected to cause "spontaneous transient inward currents," or STICs. STICs were not observed in smooth muscle cells from cerebral arteries.
25. A single Ca^{2+} spark at its peak occupies about 7 fl with an average Ca^{2+} of about 300 nM, and occurs in 0.7% of the volume of a smooth muscle cell. A Ca^{2+} spark would thus have an insignificant effect on spatially averaged $[Ca^{2+}]_i$. The highest STOC rate that we observed was 9/s, which would also have little effect on $[Ca^{2+}]_i$, assuming that this corresponded to 9 sparks/s. Even if the increase of $[Ca^{2+}]_i$ lasted 100 ms, the average Ca^{2+} in the cell would still change by <2 nM.
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28. Our results are consistent with the ryanodine- and thapsigargin-induced constrictions of the artery being caused by membrane depolarization, which increases Ca^{2+} entry through voltage-dependent Ca^{2+} channels (*3*) (Fig. 4D), particularly because the iberiotoxin-, ryanodine-, and thapsigargin-induced constrictions were similar and not additive. In support of this mechanism, we observed that ryanodine and thapsigargin had no effect on arterial diameter in the presence of the Ca^{2+} channel blocker, nisoldipine (n = 6). Further, nifedipine has been shown to block ryanodine- and cyclopiazonic acid–induced increases in spatially averaged $[Ca^{2+}]_i$ and constrictions of myogenic skeletal muscle arterioles [J. Watanabe *et al.*, *Circ. Res.* **73**, 465 (1993)].
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Localization of Protein Implicated in Establishment of Cell Type to Sites of Asymmetric Division

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Asymmetric division in *Bacillus subtilis* generates progeny cells with dissimilar fates. SpoIIE, a membrane protein required for the establishment of cell type, was shown to localize near sites of potential polar division. SpoIIE initially localizes in a bipolar pattern, coalescing at marks in the cell envelope at which asymmetric division can take place. Then, during division, SpoIIE becomes restricted to the polar septum and is lost from the distal pole. Thus, when division is complete, SpoIIE sits at the boundary between the progeny from which it dictates cell fate by the activation of a cell-specific transcription factor.

A fundamental challenge in developmental biology is to understand how cells of one type differentiate into other, more specialized types of cells (*1*). One way specialization occurs is by asymmetric cell division in which a progenitor cell gives rise to two dissimilar progeny that follow different pathways of differentiation. A simple system in which the relation between cell fate and asymmetric division has been investigated is spore formation in *Bacillus subtilis* (*2*). Spore formation involves an asymmetric cell division in which a septum is formed near one pole of the developing cell (the "sporangium"), partitioning it into unequal-sized progeny called the forespore (the small cell) and the mother cell. Crucial to the establishment of the dissimilar fates of the progeny is a putative integral membrane protein (*3*) called SpoIIE (*4*), whose synthesis commences shortly before asymmetric division (*5*).

SpoIIE is not needed for the formation of the polar septum (*6*, *7*) but is required for the activation in the forespore of a transcription factor called σ^F (*4*). The σ^F factor is present in the predivisional sporangium but is held in an inactive complex prior to septation by the inhibitory protein SpoIIAB (*8*). After the polar septum is formed, σ^F continues to be held in an inactive complex in the mother cell, while SpoIIE triggers the release of σ^F from SpoIIAB in the forespore. The mechanism by which SpoIIE activates σ^F has

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