Intracranial-Extracranial Differences in the Ca^{2+} Sensitivity of Rabbit Arteries

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Abstract. **We have previously demonstrated that the ratio of calcium uptake to force production varies widely with age, artery size, and method of contraction in cerebral arteries. The present experiments were conducted to examine the possibility that these differences involve corresponding variations in contractile force-calcium relations. Common carotid (COM), basilar (BAS), and middle cerebral (MCA) arteries from adult were denuded of endothelium and mounted *in vitro* for measurement of contractility. Following equilibration at optimum resting diameter, the arteries were permeabilized (β-escin, 50 μg/ml) and depleted of intracellular Ca^{2+} by treatment with 1 μ*M* A23187. Ca^{2+} depletion was verified by absence of any contracile response to either 25 m*M* caffeine or 1 μ*M* inositol 1,4,5-trisphosphate. Then, in the continuous presence of 1 μ*M* calmodulin, bath calcium concentration was raised from zero through 10 μ*M* in half-log increments and the corresponding contractions were recorded. For all permeabilized arteries, the maximum force produced by 10 μ*M* Ca was greater than or equal to that produced by 120 m*M* potassium-Krebs in the same segment before skinning. The pD2 (−log ED_{50}) values for calcium averaged 6.39 ± 0.03, 6.77 ± 0.04, and 6.92 ± 0.03 in COM, BA, and MCA segments, respectively. In arteries contracted by a constant submaximal concentration of calcium (0.1 μ*M* for BAS and MCA, 0.3 μ*M* for COM), the addition of 5HT produced a dose-dependent and GDPβS-sensitive increase in tension of up to 44% maximum. GTPγS mimicked the effects of 5HT and prevented further increases in Ca force induced by 5HT. Together, these data demonstrate that cerebrovascular calcium sensitivity is an anatomically heterogenous, physiologically regulated parameter responsive to agonist-induced perturbations.** [P.S.E.B.M. 1997, Vol 214]

Intracellular calcium concentration is well established as a main determinant of vascular smooth muscle contractile force. Its elevation is the trigger for contraction in response to many different neurohumoral stimuli. Intracellular calcium concentration, however, is not the only determinant of force because the latter is also dependent on the sensitivity of the myofilaments to Ca^{2+} (for review see Ref. 1). Recent studies of vascular transmembrane signaling have demonstrated that the association of agonists to their receptors can activate guanosine 5′-triphosphate (GTP)–binding proteins (G proteins), which in turn cause increased force production in absence of changes in intracellular Ca^{2+} concentration as a result of an increased Ca^{2+} sensitivity (2).

Agonist-induced Ca^{2+} sensitization of smooth muscle myofilaments can be studied in blood vessels permeabilized with α-toxin or β-escin (2, 3). In such preparations, receptor-effector coupling remains functionally intact while the ionic composition, in particular Ca^{2+} concentration, may be clamped or controlled and small molecules such as nucleotides may be diffused into the cytosol of the smooth muscle cells. Using this approach, several authors have provided direct evidence that Ca^{2+} sensitivity and its regulation by agonists vary significantly depending on vessel caliber, energetic balance or pathological status (4, 5). Together, these studies suggest that Ca^{2+} sensitivity is an independent

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mechanism for regulation of vessel contractility that may either augment or attenuate the effects of changes in cytosolic calcium concentration.

To our knowledge, no studies of calcium sensitivity have yet been performed using cerebral arteries. However, a variety of indirect evidence suggests that calcium sensitivity may vary among different cranial arteries and may contribute to differences in cerebrovascular reactivity (6). Thus, the present studies were carried out to test the hypothesis that calcium sensitivity varies among cranial arteries of differing size and can be modulated by agonist-activated G proteins. To evaluate this hypothesis, we examined calcium sensitivity under basal conditions, and during its possible modulation by G protein activation. To activate G protieins, we used the well-known G protein–coupled cerebrovascular spasmogen, 5-hydroxytryptamine (5HT).

Material and Methods

Preparation of Calcium Buffer Solutions. Calcium buffer solutions were prepared by solving the multi-equilibrium equations for interactions among the following ions: H^+, Ca^{2+}, EGTA-4, Mg^{+2}, MgATP-2, K^+, Na^+, $HEGTA^{-3}$, $H2EGTA^{-2}$, $H3EGTA^-$, H4EGTA, $CaEGTA^{-2}$, $HEGTA^-$, $MgEGTA^{-3}$, $MgHEGTA^{-1}$, ATP^{-4}, $HATP^{-3}$, $H2ATP^{-2}$, $CaATP^{-2}$, $CaHATP^-$, $KATP^{-3}$, $K2ATP^{-2}$, $KHATP^{-2}$, $NaATP^{-3}$, $Na2ATP^{-2}$, and $NaHATP^{-2}$. Equilibrium constants for these calculations were taken from previously published studies (7–9). Two main buffer solutions were prepared, one with zero free calcium (relaxing solution) and (in m*M*) 5 EGTA, 5 ATP, 110 potassium acetate, 6 Mg-acetate, 1 dithiothreitol (DTT), 0.01 leupeptin, 20 imidazole, at pH 6.8 (titrated with KOH). The other buffer solution was prepared to contain the same concentrations for all ions except for free calcium, which was 10 μ*M*. Solutions containing intermediate free calcium concentrations were prepared by mixing the high- and low-calcium buffer solutions. To verify the accuracy of these calcium buffer solutions, free calcium concentrations were measured directly in solution using dual wavelength measurements of bound to unbound fura-2 ratios at 25°C using a Jasco CAF-110 flurometer. Calcium concentrations were calculated as described by Grynkiewicz *et al.* (10). The value of the fura-2 affinity constant used was 161 n*M* for a temperature of 25°C (10).

Preparation of Arterial Rings. New Zealand white rabbits of either sex (2.0–2.8 kg) were anesthetized with sodium pentobarbital (60 mg/kg, iv) and decapitated. Segments of common carotid artery (COM), basilar artery (BAS), and middle cerebral artery (MCA) were withdrawn and placed in a Krebs' solution which contained (in m*M*): 122 NaCl, 25.6 $NaHCO_3$, 5.56 dextrose, 5.17 KCl, 2.49 $MgSO_4$, 1.60 $CaCl_2$, 0.114 ascorbic acid, and 0.027 EGTA and was continuously bubbled with 95% O_2–5% CO_2. Arteries were cleaned of adhering tissues and cut into segments approximately 2–3 mm long. Each segment was mounted on wires and suspended between a force transducer and a post attached to a micrometer as previously described (11). During all contractility experiments, the contractile tensions were continuously digitized, normalized, and recorded using an on-line computer. To avoid any possible endothelium-mediated effects, we removed the endothelium by rotating each arterial segment around the mounting wires several times to gently scrape the entire luminal surface. The loss of endothelium produced by this method was verified by the absence of a vasodilator response to 1 μ*M* acetylcholine.

After equilibration at 37°C for at least 60 min in the Krebs' solution, we obtained micrometer readings, and thereby diameter measurements (D_{zero}) for each segment under unstressed conditions at approximately 10 mg tension. Inside vessel diameter was calculated as twice the distance between the two wires divided by π. We than carried out a series of contractions in which we varied baseline stretch in regular increments from 1.2 to 3.5 times D_{zero} as previously described (11). At each level of baseline stretch, the arteries were contracted with an isotonic potassium Krebs solution containing 122 m*M* KCl and 31 m*M* NaCl, and after peak tension was reached we returned the arteries to normal sodium Krebs solution until baseline levels of tension were reestablished. Then, we incremented baseline tension and repeated the contraction cycle. The diameter associated with the development of the greatest active tension was designated as optimal resting diameter (D_{opt}). Following determination of D_{opt}, all subsequent measurements were performed at this degree of stretch.

Permeabilization. After equilibration at optimal baseline stretch, the arterial segments were placed into relaxing solution that contained (in m*M*) 5 EGTA, 5 ATP, 110 K acetate, 6 MG-acetate, 1 dithiothreitol (DTT), 0.01 leupeptin, 20 imidazole, at pH 6.8 (titrated with KOH). Chemical skinning was achieved by adding to the relaxing solution 50 μg/ml β-escin followed by incubation for 30 min at 24–25°C. The skinning solution was then replaced with relaxing solution containing 1 μ*M* A23187, in which the arteries were incubated for 20 min at 25°C to deplete the sarcoplasmic reticulum of calcium. Depletion was verified by complete absence of contractile responses to either caffeine (25 m*M*) or inositol 1,4,5-trisphosphate (IP3, 1 μ*M*). Following Ca^{2+} depletion, the vessel preparations were equilibrated for 15 min in relaxing solution containing 0.1 m*M* EGTA in order to minimize diffusion times in high EGTA buffers. Following this step, all administered solutions contained 1 μ*M* calmodulin to maintain normal responsiveness of the contractile apparatus to Ca^{2+} (3, 4).

Experimental Protocol. Contractile responses of permeabilized artery rings were recorded during sequential administrations of calcium buffer solutions with increasing free calcium concentrations between 0.03 and 10 μ*M*. Following completion of the calcium dose-response protocol, the arteries were returned to relaxing solution and once again depleted of calcium. Then, to test the effects of 5HT on calcium sensitivity, the arteries were exposed to a submaximal concentration of free calcium. We used 0.3 m*M* Ca^{2+} as submaximal concentration for COM and 0.1 m*M* Ca^{2+} for BAS and MCA. These concentrations of Ca^{2+} provided similar submaximal contractile tensions for all arteries studied. To provide a sufficient concentration of intracellular GTP, which is essential for development of receptor-mediated increases in Ca^{2+} sensitivity (1–3, 5), 100 μ*M* GTP was added in Ca^{2+}-EGTA buffer, and all further measurements were performed in the continued presence of this agent. Once the Ca^{2+}-induced contractions had stabilized, graded concentrations of 5HT (0.1–10 μ*M*) were added. After responses to 5HT had stabilized, the arteries were again returned to relaxing solution and depleted of calcium. Next, the role of G proteins in the serotonergic effects on calcium sensitivity were examined by exposing the arteries to a submaximal concentration of free calcium, followed by the addition of 1 μ*M* 5HT, then followed by addition of 1 m*M* guanosine-5′-*O*-2-thiodiphosphate (GDPβS). The arteries were then once again returned to relaxing solution, depleted of calcium, contracted by exposure to a submaximal concentration of free calcium then treated with 100 μ*M* guanosine-5′-*O*-3-thiotriophospate (GTPγS) followed by 10 μ*M* 5HT. The rundown of these preparations was negligible as indicated by the fact that three consecutive contractions to submaximal calcium concentrations could routinely be made in each preparation with no significant differences in developed tension. All preparations in which the magnitudes of consecutive contractions to submaximal calcium varied by more than 10% were excluded from the final analysis.

Data Analysis and Statistics. All values are given as mean ± SEM. In all cases, *n* refers to the number of vessel segments studied. The calcium dose-response data were fitted to the logistic equation using computerized nonlinear regression to calculate pD2 and Hill coefficient values. The effects of 5HT, GDPβS, and GTPγS on calcium-induced tension were calculated as percent changes from initial tension. The calcium pD2 data, the effects of GDPβS, and the effects of GTPγS were analyzed using a one-way analysis of variance (ANOVA) with artery type as the main factor. The effects of 5HT on calcium-induced tension were analyzed using a two-way ANOVA with artery type as one factor and 5HT concentration as the other. The correlation between calculated free calcium concentrations and fura-2 measured free calcium concentrations was determined by linear regression.

Drugs. Caffeine, EGTA, 5HT, DTT, calmodulin, GTP, GTPγS, GDPβS, and fura-2 were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade commercially available.

Results

From 14 rabbits, a total of 33 artery rings were taken for use in these studies. The relation between pre-stretch and active tension exhibited a typical parabolic shape in all three artery types examined (Fig. 1). The optimum values of the D/D_{zero} ratio averaged 2.28 ± 0.17 the MCA, 2.33 ± 0.21 for the BA, and 2.23 ± 0.08 for the COM. The values of optimal stretch are similar to those previously obtained for cranial arteries (11).

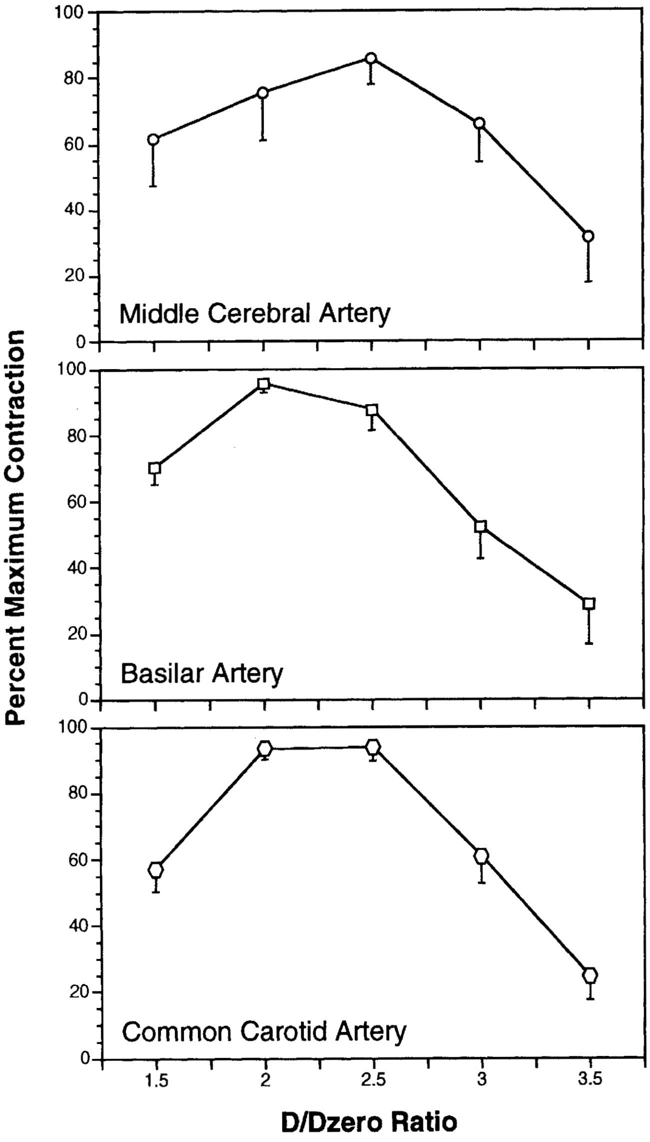

Figure 1. Length-Tension relations in rabbit cranial arteries. Each artery segment used was first repeatedly contracted with isotonic 120 m*M* potassium Krebs at varying degrees of stretch, expressed here as the D/D_{zero} ratio (abscissa). D_{zero} was defined as the minimum artery diameter measured at near-zero resting tension (≈10 mg). Contractile responses (ordinates) were normalized relative to the maximimum force observed at any amount of stretch. Vertical error bars indicate standard errors for the middle cerebral artery (upper panel, *n* = 6), basilar artery (middle panel, *n* = 9), and common carotid artery (lower panel, *n* = 12).

Intact vessel preparations were insensitive to alterations in the bath Ca^{2+} concentration before permeabilization, whereas after permeabilization with β-escin the arteries responded to Ca^{2+} in concentration-dependent manner (Fig. 2). Maximum contractile tensions induced by 10 μM Ca^{2+} after permeabilization averaged 108.2% ± 3.4%, 97.3% ± 8.9%, and 120.0% ± 10.1% of the maximum tensions induced by 120 m*M* potassium Krebs before permeabilization

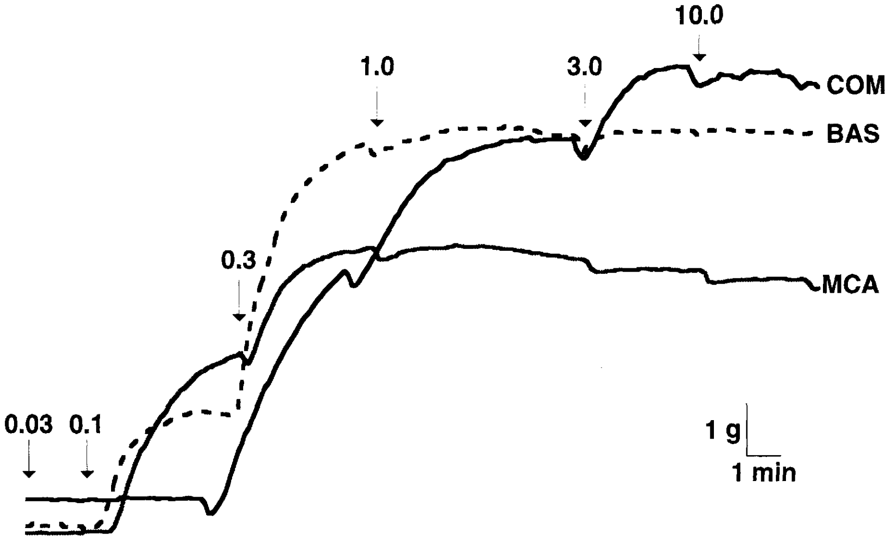

Figure 2. Representative traces of responses to calcium. Segments of rabbit common carotid (COM), basilar (BAS), and middle cerebral (MCA) arteries were permeabilized with β-escin then exposed to buffers containing varying concentrations of calcium, as indicated by the vertical arrows. All solutions contained 1 μ*M* calmodulin. The numbers above the arrows indicate calcium concentrations in μ*M*.

in COM, BAS, and MCA, respectively. Application of 120 m*M* potassium Krebs produced no contractile response in any artery after treatment with β-escin. These data indicate that the majority of cells were permeabilized by β-escin.

Figure 3 summarizes the relative force-pCa curves for each of the arteries examined. The curves for both the MCA and BAS were significantly shifted to the left in comparison to that for the COM. Correspondingly, calcium pD2 values were significantly higher for BAS and MCA than for COM (Table 1). Hill values were not significantly different among any of the arteries examined.

When the permeabilized segments were exposed to 0.3 (COM) or 0.1 (BAS and MCA) μM calcium, contractions amounting to 43.5% ± 2.9%, 41.6% ± 2.3%, and 44.2% ± 1.8% of the maximum response to calcium ensued, respectively. Subsequent addition of 5HT in the continued presence of GTP enhanced submaximal Ca^{2+}-activated force in a concentration-dependent manner (Fig. 4). Although the degree of increase in force appeared to be more marked in BAS and MCA than in COM, the magnitude of this effect was not significantly different among the three artery types.

The effect of 5HT on Ca^{2+}-activated force was reversible and was inhibited by GDPβS, a nonhydrolyzable analog of GDP that reversibly binds to G proteins and prevents their activation (3–5). As indicated in Figure 5A, the addition of GDPβS after 5HT led to a fall in force toward pre-5HT levels of Ca^{2+}-activated submaximal force for all three types of arteries studied. The average inhibitory effect of GDPβS on 5HT-induced enhancement of Ca^{2+}-activated force was 108.3% ± 16.0%, 80.1% ± 20.8%, and 91.4% ± 18.7% for COM, BAS, and MCA respectively. Addition of GDPβS alone did not depress submaximal Ca^{2+}-activated force (not shown).

To test whether the Ca^{2+} sensitizing effect of 5HT could be mimicked by GTPγS, a nonhydrolyzable analog of GTP that activates G proteins, GTPγS was added prior to 5HT in a separate series of measurements. GTPγS (100 μ*M*) modified neither resting force nor maximal force but significantly enhanced submaximal Ca^{2+}-activated force in all artery types studied (Fig. 5B). When expressed relative to the initial level of Ca^{2+}-activated force, the enhancement produced by GTPγS averaged 70.6% ± 13.2%, 109.8% ± 37.6%, and 68.1% ± 12.7% for COM, BAS, and MCA respectively; no significant differences among these means were detected. Interestingly, the effect of GTPγS was greater than, though not significantly different from, the 5HT-induced enhancement of Ca^{2+}-activated force in all three artery types. Most importantly, in the presence of 100 μ*M* GTPγS, 5HT (10 μ*M*) did not produce any additional enhancement of force in any artery examined (Fig. 5B).

Discussion

The major finding of this study is that the sensitivity of the contractile apparatus to calcium varies along the cranial vascular tree. In particular, basilar and middle cerebral arteries appeared more sensitive to calcium than the larger extracranial common carotid artery. Furthermore, Ca^{2+} sensitivity of the contractile apparatus was increased following treatment with 5HT or GTPγS, indicating that calcium sensitivity in these arteries is agonist modulated. Thus, in cranial arteries, as has been shown in arteries from other vascular beds (1–5), calcium sensitivity can be regulated by G-protein activation.

A key prerequisite for any measurement of contractile apparatus calcium sensitivity is a preparation in which cytosolic calcium concentration can be precisely controlled but normal receptor-effector mechanisms remain intact. Although a wide variety of treatments have been used to permeabilize smooth muscle, including detergents such as Triton X-l00 and saponin, many of the treatments damage either intracellular organelles or recrptor-effector

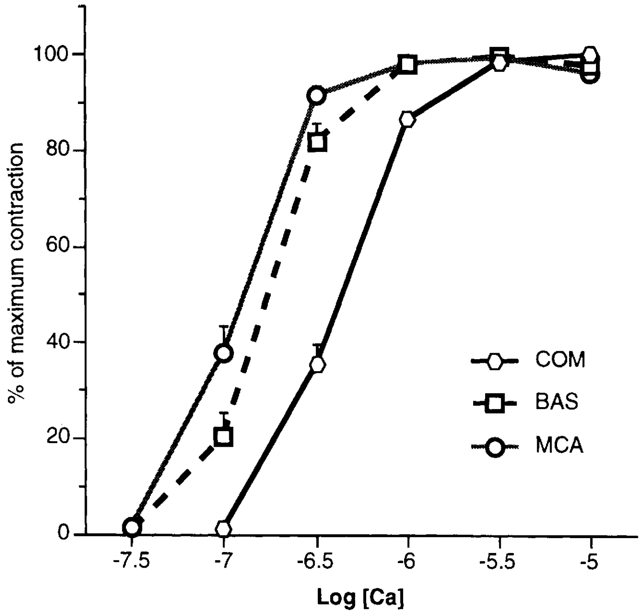

Figure 3. Calcium-force relations in β-escin–skinned rabbit cranial arteries. Shown here are the averaged calcium-force relations for β-escin–skinned common carotid (COM, *n* = 14), basilar (BAS, *n* = 12), and middle cerebral (MCA, *n* = 7) arteries. Vertical error bars indicate standard errors.

mechanisms (1). In contrast, smooth muscle preparations permeabilized with β-escin or α-toxin generally enable control of cytosolic calcium with minimal damage to normal receptor-effector mechanisms (3, 5). The present studies represent a first application of β-escin permeabilization to the study of cerebrovascular calcium sensitivity. Because the preparations used in these studies produced equivalent levels of maximum contractile tension before and after β-escin permeabilization, the treatment employed appears to have permeabilized a majority of smooth muscle cells without damage to the contractile apparatus.

A second prerequisite for studies of contractile apparatus calcium sensitivity is a system of calcium buffers which can enable precise control of cytosolic calcium concentration. Owing to the large number of calcium binding interactions in physiological buffers, submicromolar concentra-

Table I. Dose-Response Characteristics of Ca^{2+}-Induced Force in Rabbit Cranial Arteries

Artery	pD2 for Ca^{2+}	Hill coefficient
Common carotid artery (*n* = 14)	6.39 ± 0.03	2.52 ± 0.17
Basilar artery (*n* = 12)	6.77 ± 0.04[a]	3.62 ± 0.61
Middle cerebral artery (*n* = 7)	6.92 ± 0.03[a]	2.81 ± 0.29

Note. Summarized here are the average pD2 and Hill coefficient values obtained by fitting the calcium-force dose-response data with the logistic equation. The values are given as mean ± SEM for the numbers of segments indicated in parentheses.

[a] Values significantly different than those of the common carotid artery. No significant differences were observed between basilar and middle cerebral arteries for either variable.

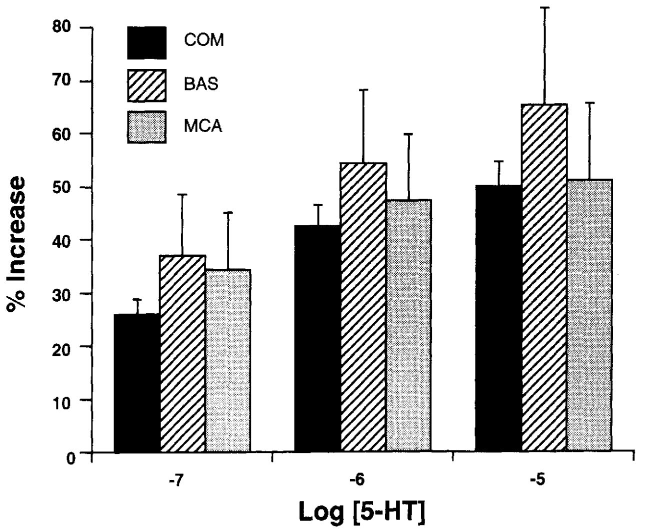

Figure 4. Effects of 5HT on calcium-induced force in β-escin–permeabilized arteries. Segments of common carotid (COM, *n* = 13), basilar (BAS, *n* = 11), and middle cerebral (MCA, *n* = 7) arteries were premeabilized with β-escin then contracted with a submaximal concentration of Ca^{2+} in the presence of 100 μ*M* GTP. Then, varying concentrations of 5HT (abscissa) were added and the percent increases in initial tension were recorded (ordinate). Effects of 5HT were calculated as percentage of initial contraction in response to submaximal concentrations of Ca^{2+}. Vertical error bars indicate standard errors.

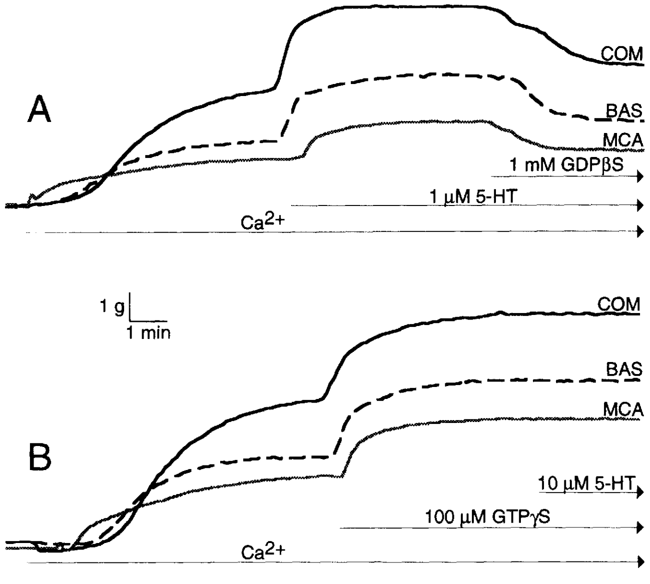

Figure 5. Effects of GDPβS and GTPγS on 5HT-induced increases in force. (A) Permeabilized segments of common carotid (COM), basilar (BAS), and middle cerebral (MCA) arteries were contracted with a submaximal concentration of Ca^{2+} (bottom horizontal arrow) then further contracted with 1μ*M* 5HT (middle horizontal arrow). Subsequent addition of 1 m*M* GDPβS (top horizontal arrow) then reduced force in all three artery types. (B) Permeabilized artery segments were contracted with a submaximal concentration of Ca^{2+} (bottom horizontal arrow) then further contracted with 100 μ*M* GTPγS (middle horizontal arrow). Subsequent addition of 10 μ*M* 5HT (top horizontal arrow) produced no additional force in any of the three artery types.

tions of calcium can be difficult to predict (7, 8). To minimize such problems, we included equilibrium constants for interactions of calcium with all known ions in our buffer systems when we calculated buffer calcium concentrations. Using our system of permeabilization and calcium buffers, we obtained highly reproducible calcium-force curves in all three arteries studied (Figs. 2 and 3). Most importantly, the relative positions of these curves varied along the cerebrovascular tree, demonstrating that the dependence of force on calcium concentration was left-shifted in the more distal cerebral arteries. Correspondingly, analysis of variance revealed that the pD2 values from these curves were significantly higher in basilar and middle cerebral arteries, than in common carotids (Table 1). There were no statistically significant differences in the Hill coefficient values, suggesting that the kinetics of the rate-limiting step coupling calcium to the development of contractile force probably were not significantly different in the three artery types examined. Because each artery used in these measurements was studied at its optimal length, as verified by independent length-tension studies (Fig. 1), the observed differences in calcium pD2 cannot be attributed to different levels of baseline stretch.

In addition to differences among artery types, calcium sensitivity also varied in response to treatment with 5HT. Because this effect was dose-dependent, the data suggest that receptor-dependent mechanisms can modulate calcium sensitivity in cerebral arteries, as has been demonstrated in arteries from other vascular beds (1). Indeed, a broad variety of different investigations suggest that muscarinic and α-adrenergic agonists, 5HT, and thromboxane A_2 analogs can all modify the sensitivity of the smooth muscle regulatory or contractile apparatus to Ca^{2+} (3, 5, 12–14). The ability of certain agonists to increase Ca^{2+} sensitivity has also been suggested by the greater force/Ca^{2+} ratios observed during agonist-induced contractions (13, 15, 16). The findings of present study confirm this phenomenon in cranial arteries for the first time and demonstrate that 5HT produces additional reversible contractions at constant submaximal concentrations of Ca^{2+} in the three artery types studied. Given that 25 m*M* caffeine and 1 μ*M* IP3 both failed to elicit any contractile response in our β-escin permeabilized preparations, it is unlikely that the observed effects of 5HT were attributable to the release of intracellular calcium. Similarly, because cytosolic Ca^{2+} concentrations in these preparations were controlled by 5 m*M* EGTA-Ca^{2+} buffers, the possibility that the 5HT effect was due to Ca^{2+} influx can also be excluded. Thus the present data strongly suggest that 5HT increases myofilament Ca^{2+} sensitivity in cerebrovascular smooth muscle cells through a receptor-dependent mechanism.

Recent reports suggest that G proteins are involved in agonist-induced increases in Ca^{2+} sensitivity (1–5, 13, 14). Consistent with these findings, 5HT enhanced Ca^{2+}-induced contractions in the present study only in the presence of GTP; in the absence of GTP the effect of 5HT was either absent or very small. These data are consistent with the results obtained by Seager et al. (17) in rabbit mesenteric artery. Furthermore, the 5HT effect was reversed by GDPβS, a competitive inhibitor of G proteins. Finally, GTPγS, a nonhydrolyzable analog of GTP that nonspecifically activated all heterotrimeric G proteins, also markedly enhanced Ca^{2+}-induced contraction. This effect of GTPγS paralleled the effect of 5HT on calcium sensitivity and eliminated the ability of 5HT to further enhance contractile force. Together, these results strongly indicate that both 5HT and GTPγS modulate calcium sensitivity *via* the same population of G proteins.

Because the relative magnitude of the 5HT- and GTPγS-mediated increases in calcium sensitivity did not vary among the three artery types studied, the present results suggest that, in contrast to baseline levels of Ca^{2+} sensitivity, the extent of agonist-induced enhancement is similar across all arteries. Thus, the observed between-artery differences in Ca^{2+} sensitivity (Fig. 3) probably involve differenced downstream from G-protein activation. It remains possible that such differences involve variations in the contractile apparatus. At present, it is well established that the sensitivity of contractile elements to Ca^{2+} is dependent on the extent of phosphorylation of the myosin light chain and is therfore closely associated with the activities of several different kinases (especially protein kinase C), phosphatases, and actin regulatory proteins (calponin, caldesmon) (18, 19). The biochemical characteristics of this system are highly variable among different types of smooth muscle cells and different vascular regions. For example, dramatic variations have been observed among myosin light chain isozyme patterns (4), isoforms of protein kinase C (20), the rate of calponin and caldesmon physphorylation (21), and the ratio of myosin kinase/phosphatase activities (19) in different vascular preparations. All these factors may modify myofilament Ca^{2+} sensitivity, although their exact role in the observed differences of Ca^{2+} sensitivity between intra- and extracranial arteries has yet to by clarified.

Previous studies have demonstrated that contractions in cerebral arteries are more dependent on Ca^{2+} fluxes through voltage-operated gates in the plasma membrane than are arteries from other vascular beds (22, 23). In addition, small cerebral arteries are more dependent than large arteries on calcium influx from the extracellular space to sustain contraction as suggested by the finding that nifedipine-induced dilatory response of intracerebral vessels increased significantly with decreasing arterial size (24). Consistent with these findings, the magnitude of calcium uptake was significantly greater in middle cerebral than in common carotid arteries during contractions in reponse to either potassium or 5HT (6). These differences can be attributed, at least in part, to a lower content of sarcoplasmic reticulum in small arteries (25). Hypothetically, increased Ca^{2+} uptake may compensate for the relative paucity of intracellular Ca^{2+} stores in intracranial arteries. The present results further suggest that differences in calcium sensitivity may also offset the effects of small intracellular stores of calcium and participate in the regulation of contractility in intracranial arteries.

Overall, the present data suggest a novel mechanism to explain differences in cerebrovascular reactivity at different levels of the cerebrovascular tree. Together these finding reflect the importance of balance between dependence on intracellular calcium release, extracellular calcium entry, and changes in calcium sensitivity in the overall regulation of cerebrovascular contractility. Although the functional significance and mechanisms of variations in calcium sensitivity have yet to be clairifed, it seems probable that modulation of contractile protein Ca^{2+} sensitivity contributes in an important and artery-size–dependent manner to regulation of cerebral blood flow.

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