Effects of Calcium Antagonists on Isolated Bovine Cerebral Arteries: Inhibition of Constriction and Calcium-45 Uptake Induced by Potassium or Serotonin

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The purpose of this study was to determine the mechanisms by which organic calcium channel blockers inhibit cerebral vasoconstriction. Isolated bovine middle cerebral arteries were cut into rings to measure contractility or into strips to measure radioactive calcium (45Ca) influx and efflux. Calcium channel blockers (10^-5 M verapamil or 3.3 x 10^-7 M nifedipine) and calcium-deficient solutions all produced near-maximal inhibition of both potassium- and serotonin-induced constriction. In calcium-deficient solutions containing potassium or serotonin, verapamil and nifedipine each blocked subsequent calcium-induced constriction in a competitive manner. Potassium and serotonin significantly increased 45Ca uptake into cerebral artery strips during 5 minutes of 45Ca loading; for potassium 45Ca uptake increased from 62 to 188 nmol/g, and for serotonin from 65 to 102 nmol/g. Verapamil or nifedipine had no effect on basal 45Ca uptake but significantly blocked the increase in 45Ca uptake induced by potassium or serotonin. Potassium, and to a lesser extent serotonin, each induced a brief increase in the rate of 45Ca efflux into calcium-deficient solutions. Verapamil or nifedipine had no effect on basal or potassium-stimulated 45Ca efflux. The results demonstrate that verapamil and nifedipine block 45Ca uptake through both potential-operated (potassium) and receptor-operated (serotonin) channels in bovine middle cerebral arteries.

The mechanism by which organic calcium channel blockers (CCBs) inhibit receptor-mediated cerebral vasoconstriction is controversial. CCBs or Ca2+-deficient solutions inhibit serotonin-induced constriction by isolated cerebral arteries from humans, and many experimental animals, including monkeys, dogs, goats, rabbits, and cattle. These investigations suggest, albeit indirectly, that receptor-mediated agents such as serotonin (5-HT) constrict cerebral arteries mainly by promoting the influx of extracellular calcium through receptor-operated channels sensitive to CCBs. However, the few direct studies with radioactive calcium-45 (45Ca) suggest that receptor-mediated agents constrict cerebral arteries by releasing Ca2+ from intracellular stores, and that the Ca2+ release can be blocked by CCBs. 5-HT-induced constriction, although inhibited by CCBs, was not accompanied by a measurable increase of 45Ca uptake into bovine basilar arteries. CCBs can enter into smooth muscle and have been shown to inhibit 45Ca flux into a sarcoplasmic reticulum fraction isolated from rabbit cerebral arteries.

The purposes of the present study were 1) to measure basal 45Ca fluxes in isolated cerebral arteries, and 2) to determine the effects of 2 CCBs (verapamil and nifedipine) and 2 vasoconstrictors (K+ and 5-HT) on cerebrovascular constriction and 45Ca fluxes.

Materials and Methods

Solutions and Drugs

The experiments employed bicarbonate-buffered solutions (BBSs) aerated with 95% O2 and 5% CO2. BBS contained (in mM) 122.5 NaCl, 4.0 KCl, 1.2 MgSO4·7H2O, 1.2 KH2PO4, 1.2 CaCl2·2H2O, 17.5 NaHCO3, 10 glucose, and 0.04 ethyleneglycol-bis(β-aminoethylether) N,N'-tetraacetic acid (EGTA). For K+-rich BBS, 122.5 mM KCl and 17.5 mM KHCO3 were isosmotically substituted for NaCl and NaHCO3. Ca2+-deficient solutions had no CaCl2 added. Concentrated HCl, NaOH, or KOH was used to adjust the pH of the solutions to 7.4. Solutions were prepared with deionized water; chemicals were of analytic grade.

Serotonin (5-hydroxytryptamine creatinine sulfate) was purchased from Sigma, 4Ca from ICN Radiochemicals, and [3H]sorbitol from New England Nuclear. Verapamil HCl (Knoll Pharmaceuticals) and nifedipine (Bayer) were generously provided by the respective manufacturers. Concentrated solutions of the agents were prepared in deionized water, except for nifedipine, which was dissolved in dimethylsulfoxide (DMSO) (57 µg nifedipine/ml DMSO). Solutions were freshly prepared; foil-wrapped containers prevented light degradation.

Tissue Material

Bovine middle cerebral arteries (BMCAs) were obtained from freshly slaughtered animals.
ing Company, Mainland, Penn.). BMCAs were isolated, immersed in BBS at 1°C, transported to the laboratory, and cleaned.

**Contractility Experiments**

BMCAs were cut into rings of uniform width (2.5 mm). Each ring was mounted vertically on two L-shaped stainless steel wire holders (0.04 mm o.d.) between a glass anchor and a Statham isometric transducer. The rings were maintained in 25–50 ml of oxygenated BBS (37°C, pH 7.4) over a 2–3 hour equilibration period. Resting tension was gradually increased to 0.5 g, which was optimal for rings of BMCAs according to the method of Högestätt et al. Isometric tension development was recorded on digital readout amplifiers and on Kipp and Zonen Model BD40 and Gould Brush Model 816 recorders.

**Preparation for 45Ca Flux Experiments**

BMCAs were opened into strips approximately 1.5 cm long. Each strip was quickly blotted on filter paper, weighed, mounted on a stainless steel wire holder, and allowed to equilibrate in BBS at 37°C for 3 hours. The radioactive bicarbonate-buffered solutions (RBBSs) that were used to load the strips with 43Ca all contained 0.2 μCi/ml 43Ca, 0.2 μCi/ml [3H]sorbitol, and 0.1 mg% nonradioactive sorbitol.

**Determination of 45Ca Uptake**

To observe the time course of basal 45Ca uptake into BMCAs, strips were incubated in RBBS at 37°C for up to 2 hours. To determine the effects of CCBs on basal and K+-stimulated 43Ca efflux at 37°C, strips were preincubated for 45 minutes in 37°C BBS containing no drug (control), 10^{-3} M verapamil, 2 μl DMSO/ml BBS, or 3.3 × 10^{-1} M nifedipine. The strips were then incubated for 60 minutes in RBBS under the same conditions as before, or in RBBS containing 144 mM K+ or 10^{-3} M 5-HT. All strips were blotted with a Kimwipe tissue, dipped momentarily in Ca2+-deficient BBS at 37°C, blotted again, and immersed in 30 ml of ice-cold Ca2+-deficient BBS containing 2 mM EGTA (EGTA solution) for 40 minutes. The strips were incubated overnight in 3 ml of HNO3 solution either 1) immediately after procurement at the slaughterhouse; 2) after immersion in ice-cold BBS, transport to the laboratory, and weighing; 3) after reequilibration in BBS at 37°C for 3 hours; or 4) after exposure to ice-cold EGTA solution for an additional 40 minutes. Strips were blotted and dipped as previously described, and were immersed in EGTA solution at 1°C or 37°C for different time intervals during 2 hours. In the experiments at 37°C, some strips were exposed to EGTA solution containing 144 mM K+ or 10^{-3} M 5-HT during the second hour. To determine the effects of CCBs on basal and K+-stimulated 43Ca efflux at 37°C, strips were exposed to Ca2+-deficient 2 mM EGTA BBS between 0 and 60 minutes and to Ca2+-deficient K+-rich 2 mM EGTA BBS between 60 and 120 minutes. Strips were exposed to either no drug (control), 10^{-3} M verapamil, 2 μl/ml DMSO, or 3.3 × 10^{-7} M nifedipine between 15 and 120 minutes. After 2 hours, all strips were incubated overnight in 3 ml of HNO3 solution at 25°C. The 3-ml aliquots were counted for 45Ca and [3H]cpm and analyzed for 43Ca content, [3H]sorbitol space, and 43Ca uptake as previously described. The data obtained were expressed as either desaturation curves (which show the decline of strip 43Ca content with time) or as curves showing the fractional rate of 43Ca efflux (percent of 43Ca lost per minute). The fractional rate of 43Ca efflux is sensitive to nonsteady-state changes in efflux, and was calculated as follows:

\[
\text{Rate of } ^{43}\text{Ca efflux} = \frac{100 \times ^{43}\text{Ca cpm lost during time interval}}{\text{average } ^{43}\text{Ca cpm remaining in strip } \times \text{time interval}}
\]  

where cpm is the increase in cpm above blank cpm and time interval is in minutes.

**Determination of 46Ca Content**

Strips of BMCAs were prepared as in the 43Ca flux experiments. The strips were blotted, dipped in Ca2+-deficient BBS at 37°C, blotted again, and incubated overnight in HNO3 solution either 1) immediately after procurement at the slaughterhouse; 2) after immersion in ice-cold BBS, transport to the laboratory, and weighing; 3) after reequilibration in BBS at 37°C for 3 hours; or 4) after exposure to ice-cold EGTA solution for an additional 40 minutes. Strips were counted for 46Ca content (μmol/g wet wt) by measuring the absorbance at 422.7 nm of the HNO3 solutions with a Perkin-Elmer Model 2380 atomic absorption spectrophotometer.
Statistical Analysis

Rings or strips of BMCA were selected at random to receive a drug or special treatment. Data are expressed as means ± SEM, with n = 6 rings or strips from at least 5 different animals for each variable. The null hypothesis was examined using analysis of variance (ANOVA) and Newman-Keuls multiple range t tests (NK) and was rejected at p < 0.05. Calculations and statistical analyses were performed on microcomputers (Tandy TRS-80 Models III and IV) with BASIC or PHARM/PCS programs.26

Results

Contractility Experiments

Verapamil and nifedipine each produced dose-related relaxation of BMCA preconstricted with 144 mM K+; half-maximal relaxation was produced by 10⁻³ M verapamil or 7.2 x 10⁻⁹ M nifedipine. These concentrations of CCBs producing 50% relaxation of K⁺-constricted BMCA were comparable to those reported for K⁺-constricted arteries from humans1,2 and other species.27-30 Near-maximal relaxation was produced by 10⁻⁵ M verapamil and 3.3 x 10⁻⁷ M nifedipine; these concentrations of CCBs were employed in subsequent experiments. DMSO had no significant effect on these concentrations of CCBs were employed in subsequent experiments.

Calcium withdrawal produced relaxation of BMCA preconstricted with 144 mM K⁺ or 10⁻³ M 5-HT. However, there was a latency for BMCA preconstricted with 5-HT. After 5 minutes the reduction in K⁺-induced tension was 35.8 ± 6.0%, while the reduction in 5-HT-induced tension was only 5.0 ± 3.2%. Near-maximal relaxation of both K⁺- and 5-HT-induced constriction occurred at 45 minutes. Verapamil (3.3 x 10⁻⁶ M) and 3.3 x 10⁻⁷ M nifedipine also produced near-maximal relaxation of K⁺-constricted BMCA within 45 minutes so this time interval was used for pretreatment with calcium antagonists in subsequent experiments.

Control rings of BMCA developed slight active tone in BBS containing 1.2 mM CaCl₂ after the equilibration period; in 45 minutes, basal tension increased 0.13 ± 0.04 g with Ca²⁺-deficient BBS, 10⁻⁵ M verapamil, or 3.3 x 10⁻⁷ M nifedipine; these concentrations of CCBs were employed in subsequent experiments. DMSO had no significant effect on these concentrations of CCBs were employed in subsequent experiments.

Determination of ⁴⁰Ca Content

The total ⁴⁰Ca content of BMCA at the slaughterhouse was 4.0 ± 0.3 μmol/g. After isolation, transport in ice-cold BBS, and weighing, the total ⁴⁰Ca content approximately doubled, to 8.4 ± 0.5 μmol/g. Reequilibration in BBS at 37° C for 3 hours was necessary to reduce ⁴⁰Ca content to control levels (4.4 ± 0.4 μmol/g); the 3-hour equilibration period was used in subsequent experiments with ⁴⁰Ca. The residual ⁴⁰Ca content (that remaining after exposure to ice-cold EGTA solution for an additional 40 minutes) was 2.1 ± 0.5 μmol/g. EGTA-extractable ⁴⁰Ca content (that displaced from strips during the 40-minute interval) was thus calculated to be 2.3 μmol/g.

Figure 1. Effects of calcium antagonism on K⁺- and serotonin-induced constriction of bovine middle cerebral arteries (BMCA). Rings of BMCA were pretreated with no agent (control, ●), Ca²⁺-deficient BBS (○), 10⁻⁵ M verapamil (■), or 3.3 x 10⁻⁷ M nifedipine (□) for 45 minutes and were then exposed to increasing concentrations of K⁺ or serotonin. Data are expressed as percent of maximal control tension (mean ± SEM) attained by each vasoconstrictor, n = 7-9 rings of BMCA per data point. At maximal concentrations of K⁺ or serotonin, constriction by all treated rings was significantly less than constriction by controls (p<0.01, ANOVA, Newman-Keuls).

K⁺ (144 mM) and 10⁻⁴ M 5-HT produced little constriction in Ca²⁺-deficient BBS; adding 10⁻³ M verapamil or 3.3 x 10⁻⁷ M nifedipine for an additional 30 minutes had no effect on this tone. CaCl₂ produced dose-dependent constriction when it was added to the Ca²⁺-deficient BBS containing K⁺ or 5-HT (Figure 2). Verapamil or nifedipine inhibited the subsequent CaCl₂-induced vasoconstriction in a competitive manner; little constriction occurred until the calcium concentration exceeded 10⁻³ M.

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FIGURE 2. Effects of calcium antagonism on CaCl₂-induced constriction. Rings of bovine middle cerebral artery (BMCA) were exposed to Ca²⁺-deficient BBS for 45 minutes, to Ca²⁺-deficient BBS containing K⁺ or serotonin for 30 minutes, and then to no agent (control, •), 10⁻⁵ M verapamil (○), or 3.3 x 10⁻⁶ M nifedipine (□) for an additional 30 minutes. CaCl₂ was restored to the media to obtain CaCl₂ dose-response curves. Data are expressed as percent of maximal constriction (mean ± SEM) attained by CaCl₂ in the presence of each vasoconstrictor, n = 7–9 rings of BMCA per data point. At a CaCl₂ concentration of 1.2 x 10⁻³ M, constriction by all treated rings was significantly less than constriction by control rings (p<0.01, ANOVA, Newman-Keuls).

Time Course of Basal ⁴⁵Ca Uptake at 37° C
BMCA exposed to RBBS showed rapid increases in total ⁴⁵Ca content, total [³H]sorbitol space, extracellular bound ⁴⁵Ca content, and ⁴⁵Ca uptake (Figure 4). The [³H]sorbitol space achieved a steady state in 1.5, total and bound ⁴⁵Ca contents in 10, and ⁴⁵Ca uptake in 20 minutes. Calcium in the external medium did not exchange completely with ⁴⁵Ca already present in BMCA. For example, after 2 hours of ⁴⁵Ca loading, ⁴⁵Ca uptake was 0.12 ± 0.01 nmol/g, and residual ⁴⁵Ca content was 1.35 ± 0.11 nmol/g. Residual ⁴⁵Ca content did not change significantly over the 2-hour interval; the ⁴⁵Ca content at 0 minutes was 1.43 ± 0.19 nmol/g. On the basis of these ⁴⁵Ca uptake studies, in subsequent experiments strips of BMCA were loaded with ⁴⁵Ca and [³H]sorbitol for either 5 (⁴⁵Ca uptake at 37° C) or 60 minutes (⁴⁵Ca efflux at 37° C). For the first 5 minutes, basal ⁴⁵Ca uptake was approximately linear, and at 60 minutes basal ⁴⁵Ca uptake was maximal.

Effects of CCB on ⁴⁵Ca Uptake
Pretreatment with verapamil or nifedipine had no effect on control ⁴⁵Ca uptake during 5 minutes of ⁴⁵Ca loading (Figure 5). K⁺ or 5-HT significantly increased ⁴⁵Ca uptake; for K⁺, ⁴⁵Ca uptake increased from 62 to 188 nmol/g, and for 5-HT, from 65 to 102 nmol/g. Verapamil or nifedipine significantly blocked the increase in ⁴⁵Ca uptake induced by K⁺ or 5-HT; the resultant ⁴⁵Ca uptake was not significantly greater than control. DMSO had no effect on basal or on K⁺-induced ⁴⁵Ca uptake, but reduced 5-HT-induced ⁴⁵Ca uptake (p<0.05). Control total ⁴⁵Ca content was 1.37 ± 0.05 μmol/g, of which 0.31 ± 0.02 μmol/g was present in the total [³H]sorbitol space (0.25 ± 0.01 ml/g) and 1.06 ± 0.01 μmol/g was bound. Control residual ⁴⁵Ca content was 1.20 ± 0.08 μmol/g. These parameters were not significantly altered by exposure to verapamil, DMSO, nifedipine, K⁺, 5-HT, or combinations of these agents.

⁴⁵Ca Efflux Experiments at 37° C
After strips were loaded with ⁴⁵Ca for 60 minutes, exposure to Ca²⁺-deficient 2 mM EGTA BBS at 37° C produced a time-dependent reduction in ⁴⁵Ca content (Figure 6). Efflux of ⁴⁵Ca was more rapid at 37° C than at 1° C (Figure 3); for example, at 120 minutes the

0.29 ± 0.01 μmol/g was in the extracellular water, estimated by the [³H]sorbitol space (0.242 ml/g).
fractional rate of $^{45}$Ca efflux was 1.07 ± 0.03%/min at 37°C and 0.21 ± 0.02%/min at 1°C. K$^+$ or 5-HT produced a brief increase in the rate of $^{45}$Ca efflux (Figure 6). For the first 5 minutes after exposure to K$^+$, the rate of $^{45}$Ca efflux from BMCA increased 57% (2.2 ± 0.1%/min vs. 1.4 ± 0.1%/min for controls). 5-HT induced a 30% increase in the rate of $^{45}$Ca efflux (1.8 ± 0.1%/min). The increase in K$^+$-stimulated $^{45}$Ca efflux was sustained for 20 minutes, while a significant increase in 5-HT-stimulated $^{45}$Ca efflux was observed only in the first 5 minutes. Verapamil or nifedipine had no significant effect on basal or K$^+$-stimulated $^{45}$Ca efflux (Figure 7).

**Discussion**

Vascular smooth muscle (VSM) contraction depends on an increase in free cytoplasmic calcium.$^{32}$ The source of this calcium varies depending on the blood vessel studied and the agent used to produce contraction.$^{33,34}$ Agents such as K$^+$ constrict VSM via electromechanical coupling$^{35,36}$; in high concentrations, K$^+$ depolarizes the plasma membrane of VSM and promotes the influx of extracellular Ca$^{2+}$ through potential-dependent channels. Receptor-mediated agents such as norepinephrine or 5-HT promote constriction via pharmacomechanical coupling$^{35,36}$; they can promote the influx of extracellular Ca$^{2+}$ through receptor-operated channels independent of membrane depolarization, or release Ca$^{2+}$ from internal stores such as the sarcoplasmic reticulum.$^{18,33}$

In this in vitro study with BMCA, we examined the effects of verapamil and nifedipine on cerebral vasoconstriction and $^{45}$Ca fluxes. Based on radioligand binding studies, these CCBs are postulated to act at two different binding sites on Ca$^{2+}$ channels in peripheral VSM.$^{37,38}$ We also used Ca$^{2+}$-deficient solutions containing EGTA to displace extracellularly bound Ca$^{2+}$ and to block Ca$^{2+}$ influx into BMCA.

Verapamil, nifedipine, or Ca$^{2+}$ withdrawal produced slight relaxation of stretched BMCA, indicating that a small portion of the resting tension of BMCA is maintained by the influx of extracellular Ca$^{2+}$ through CCB-sensitive channels. Verapamil and nifedipine had no effect on basal $^{45}$Ca uptake into BMCA (Figure 5) in accordance with most previous studies on peripheral VSM.$^{39}$ Basal $^{45}$Ca uptake into VSM is postulated to take place via leak-operated Ca$^{2+}$ channels.$^{39}$

The contractility experiments (Figures 1 and 2) and $^{45}$Ca uptake experiments (Figure 5) in this study support the hypothesis that Ca$^{2+}$ antagonists inhibit the constriction of BMCA by blocking the influx of extracellular Ca$^{2+}$ through both potential-operated (K$^+$) and receptor-operated (5-HT) channels in the plasma membrane. The effects of verapamil and nifedipine on K$^+$-
Ca^{2+} efflux at 37°C. Bovine middle cerebral arteries were incubated in radioactive bicarbonate-buffered solution (BBS) for 60 minutes and then exposed to aliquots of 37°C Ca^{2+}-deficient 2 mM EGTA BBS (control, •) at various time intervals over 2 hours. Other strips were exposed to Ca^{2+}-deficient 2 mM EGTA BBS containing 144 mM K+ (○) or 10^{-5} M serotonin (*) for the second hour. Data are expressed as the mean percent of total 45Ca content remaining or as the mean rate of 45Ca efflux (% of 45Ca content lost/min) ± SEM, n = 18 strips (during the first 60 minutes) or 6 strips (from 60 to 120 minutes) per data point. Significant differences from untreated controls are indicated as * (p<0.05, ANOVA, Newman-Keuls).

5-HT-induced cerebral vasoconstriction, although clearly mediated through 5-HT receptors, may be due to the influx of extracellular Ca^{2+} through potential-operated channels (POC) as well as through receptor-operated channels (ROC). 5-HT depolarizes cerebral arteries from several species, notably cats, dogs, and guinea pigs. In cats and dogs there is a strong correlation between 5-HT-induced membrane depolarization and cerebral vasoconstriction. The 5-HT-induced depolarization reported in these studies may be secondary to Ca^{2+} influx through ROC since the depolarization itself can be blocked in cat middle cerebral arteries by verapamil. If 5-HT constricts cerebral arteries by electromechanical as well as pharma-

induced 4Ca uptake into BMCA are similar to those reported for cinnarizine in bovine basilar arteries and for nicardipine in guinea pig basilar artery. Takagi et al found that verapamil produced near-maximal inhibition of 5-HT-induced constriction, as in this study (Figure 1), but that 5-HT had no effect on 4Ca uptake. This discrepancy may be due to differences between basilar and middle cerebral arteries in the same species, but it is more likely to be due to differences in methodology. Takagi et al used 10 mM LaCl3 to block Ca^{2+} fluxes across the plasma membrane, but subsequent studies showed that a high concentration of LaCl3, or EGTA plus low temperatures are required to block all Ca^{2+} efflux from peripheral VSM. The 10 mM LaCl3 may have been inadequate to prevent 4Ca efflux from bovine basilar arteries after loading, causing the previous 5-HT-induced 4Ca influx to go undetected.

5-HT produced only a modest increase in 4Ca uptake compared with K+ (Figure 5). There are two possible explanations for this finding: 1) K+ produced greater constriction of BMCA than 5-HT in the concentrations employed, or 2) the external Ca^{2+} contributing to K+ and 5-HT-induced constriction may bind to different sites on BMCA. Two distinct Ca^{2+} binding sites have been demonstrated in peripheral VSM, a low-affinity Ca^{2+} binding site important for the stimulatory action of K+, and a high-affinity Ca^{2+} binding site important for the stimulatory action of norepinephrine. If the Ca^{2+} important for 5-HT-induced constriction of BMCA were more tightly bound to high-affinity Ca^{2+} binding sites, it would not exchange as readily with 4Ca in the bathing medium. This may explain why 5-HT produced less of an increase in 4Ca uptake than K+ after 5 minutes of 4Ca loading (Figure 5) and why tension loss with Ca^{2+} withdrawal was delayed when BMCA was preconstricted with 5-HT.

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![Figure 6. Effects of K+ and serotonin (5HT) on 45Ca efflux at 37°C.](image)

![Figure 7. Effects of calcium antagonists on basal and K+-stimulated 45Ca efflux at 37°C.](image)
comechanical coupling, promoting the influx of extracellular Ca\(^{2+}\) through POC as well as ROC, this may explain why 5-HT-induced constriction of cerebral arteries from cattle (Figure 1), humans,\(^5-12\) and other species\(^2-11\) is so well antagonized by CCB. POC in peripheral VSM generally exhibit a greater sensitivity to CCB than ROC, so a greater degree of recruitment of POC would be expected to cause a greater sensitivity to CCB.\(^39\)

Verapamil or nifedipine had no effect on basal \(^{43}\)Ca efflux from BMC into Ca\(^{2+}\)-deficient solutions (Figure 7), so these CCBs do not relax BMC by promoting Ca\(^{2+}\) extrusion.

Several investigators have suggested that receptor-mediated agonists, such as 5-HT, constrict cerebral or peripheral arteries by releasing Ca\(^{2+}\) from intracellular stores, and that CCBs may inhibit the release of this intracellular Ca\(^{2+}\).\(^2,3,14,53,54\) If this were the case in isolated cerebral arteries, 5-HT should increase \(^{43}\)Ca efflux, and the 5-HT-stimulated \(^{43}\)Ca efflux should be inhibited by CCB. In this study with BMC, 5-HT produced only a negligible increase in \(^{43}\)Ca efflux into Ca\(^{2+}\)-deficient solutions (Figure 6). K\(^+\) produced a greater increase in \(^{43}\)Ca efflux from BMC (Figure 6), and the K\(^+\)-stimulated efflux was not blocked by verapamil or nifedipine (Figure 7). There are two possible mechanisms to account for K\(^+\)- and 5-HT-stimulated \(^{43}\)Ca efflux from BMC:

1. These constrictors may temporarily release Ca\(^{2+}\) from intracellular stores into the cytoplasm of BMC, in which case the increase in \(^{43}\)Ca efflux represents an increase in the transport of cytoplasmic Ca\(^{2+}\) to the extracellular space. However, if this is the case, then the Ca\(^{2+}\) released into the cytoplasm of BMC by K\(^+\) and 5-HT must not be sufficient to produce constriction in Ca\(^{2+}\)-deficient solutions (Figure 1). In support of this mechanism, Kobayashi et al\(^32\) found that K\(^+\)-depolarization did not produce constriction of rat aortas preincubated in Ca\(^{2+}\)-free media containing 2 mM EGTA but did induce a transient increase in free cytoplasmic Ca\(^{2+}\) (measured by the Ca\(^{2+}\) fluorescent dye, quin 2).

2. K\(^+\) and 5-HT may release \(^{43}\)Ca from tightly bound sites on the plasma membrane directly into the extracellular space. Such a mechanism would also explain why K\(^+\) and 5-HT release \(^{43}\)Ca and yet produce no constriction in Ca\(^{2+}\)-deficient solutions. The present results suggest that K\(^+\) and 5-HT can induce Ca\(^{2+}\) release from internal stores and/or external binding sites, but that the Ca\(^{2+}\) release from BMC is temporary, cannot sustain constriction, and, at least in the case of K\(^+\), is not blocked by CCB.

In summary, K\(^-\) or 5-HT-induced constriction of BMC is inhibited by verapamil, nifedipine, and Ca\(^{2+}\) withdrawal. In Ca\(^{2+}\)-deficient media containing K\(^+\) or 5-HT, no constriction occurs until Ca\(^{2+}\) is added to the media; verapamil or nifedipine blocks this Ca\(^{2+}\)-induced constriction in a competitive manner. The results of these contractility studies with BMC are compatible with results reported by other investigators for cerebral arteries from humans\(^2-10,27-30\) indicating that BMCAs are not atypical in their response to K\(^+\), 5-HT, and calcium antagonists. Our \(^{43}\)Ca flux studies on BMC support the hypothesis that CCBs inhibit K\(^-\) and 5-HT-induced cerebrovascular constriction by blocking the influx of extracellular Ca\(^{2+}\) rather than by blocking Ca\(^{2+}\) release from internal stores. K\(^+\)-induced \(^{43}\)Ca release was not inhibited by verapamil or nifedipine, and 5-HT-induced \(^{43}\)Ca release was negligible. On the other hand, K\(^+\) and 5-HT each induced an increase in \(^{43}\)Ca uptake, which was blocked by verapamil or nifedipine. This study provides evidence that CCBs inhibit \(^{43}\)Ca uptake through both potential-dependent (K\(^+\)) and receptor-mediated (5-HT) Ca\(^{2+}\) channels in cerebrovascular smooth muscle.

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