Effects of Prostaglandin F$_{2\alpha}$ and Thromboxane A$_2$ Analogue on Bovine Cerebral Arterial Tone and Calcium Fluxes

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We determined sources of activator calcium for prostanoid-induced cerebrovascular constriction by measuring isometric tension and calcium-45 ($^{45}$Ca) fluxes in bovine middle cerebral arteries. Constriction induced by prostaglandin F$_{2\alpha}$, or the stable thromboxane A$_2$ analogue SQ-26,655 was near-maximally inhibited in calcium-deficient solutions but only partially inhibited by calcium antagonists (10$^{-5}$ M verapamil or 3.3x10$^{-7}$ M nifedipine). Studies of $^{45}$Ca binding at different external Ca$^{2+}$ concentrations showed that cerebral arteries possess two calcium binding sites, a high-affinity site and a low-affinity site. Each prostanoid significantly increased low-affinity $^{45}$Ca uptake (external Ca$^{2+}$ concentration = 1.2 mmol/l) during 5 minutes of $^{45}$Ca loading; for prostaglandin F$_{2\alpha}$, $^{45}$Ca uptake increased from 69 to 108 nmol/g and for SQ-26,655, from 78 to 141 nmol/g. The prostanoid-induced increases in low-affinity $^{45}$Ca uptake were completely abolished by pretreatment with verapamil or nifedipine. Prostaglandin F$_{2\alpha}$, SQ-26,655, verapamil, and nifedipine had no effect on high-affinity $^{45}$Ca uptake (external Ca$^{2+}$ concentration = 45 $\mu$mol/l) or $^{45}$Ca efflux (after 60 minutes' preincubation in calcium-deficient media). Prostaglandin F$_{2\alpha}$ and SQ-26,655 each appear to constrict cerebral arteries by two mechanisms: first, by promoting calcium uptake from low-affinity binding sites through receptor-operated channels sensitive to the calcium antagonists, and second, by releasing calcium from depletable internal stores. (Stroke 1991;22:66-72)

Vasoconstrictor prostanoids such as thromboxane A$_2$ (TxA$_2$) or prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) have been implicated in the pathogenesis of many cerebrovascular disorders including stroke, post-ischemic hypoperfusion after cardiac arrest, and cerebral vasospasm after subarachnoid hemorrhage.1-4 Both TxA$_2$ (released from aggregating platelets) and PGF$_{2\alpha}$ are potent constrictors of human cerebral arteries.5-9 Thromboxane B$_2$ (TxB$_2$), the stable metabolite of TxA$_2$, and PGF$_{2\alpha}$ rapidly accumulate in brain tissue after cerebral ischemia.2,10,11 Clinical studies have demonstrated elevated levels of TxB$_2$ or PGF$_{2\alpha}$ in human cerebrospinal fluid following stroke or subarachnoid hemorrhage.12-14 Organic calcium antagonists (CAs) have been used in clinical settings to confer cerebral protection after stroke or cerebral vasospasm following subarachnoid hemorrhage.15-17 However, the ability of CAs to improve cerebral blood flow in these cerebrovascular disorders may be limited if vasoactive agents such as TxA$_2$ or PGF$_{2\alpha}$ are involved in their pathogenesis.17,18 Prostanoid-induced constriction of isolated cerebral arteries is calcium-dependent but is partially resistant to inhibition by CAs such as nifedipine,5 nimodipine,5,8,19 nicardipine,20,21 diltiazem,22 or flunarizine.23 The mechanism of resistance to CAs is not known. If CAs inhibit prostanoid-induced Ca$^{2+}$ uptake through CA-sensitive channels in cerebrovascular smooth muscle,24 then CA-resistant constriction could be due to Ca$^{2+}$ uptake through CA-insensitive channels, Ca$^{2+}$ release from intracellular stores such as the sarcoplasmic reticulum, or other mechanisms.

The purpose of this study was to identify the sources of activator calcium by which TxA$_2$ and PGF$_{2\alpha}$ constrict cerebral arteries. Isolated bovine middle cerebral arteries were cut into rings to measure isometric tension or into strips to measure calcium-45 ($^{45}$Ca) fluxes directly. Calcium-deficient solutions and two prototypic CAs, nifedipine (a dihydropyridine) and verapamil (a phenylalkylamine), were used to antagonize prostanoid-induced constriction. Nifedipine and verapamil have previously been shown to inhibit po-
tassium-induced constriction and $^{45}$Ca uptake in cerebral arteries. The stable TxA$_2$ mimetic SQ-26,655 was used instead of TxA$_2$, which constricts cerebral arteries. The stable TxA$_2$ mimetic SQ-26,655 (Knoll) was used instead of TxA$_2$, which constricts cerebral arteries.25 The stable TxA$_2$ mimetic SQ-26,655 (Knoll) was used instead of TxA$_2$, which constricts cerebral arteries. 25 The stable TxA$_2$ mimetic SQ-26,655...)

Materials and Methods

Bovine middle cerebral arteries were obtained from freshly slaughtered animals. The arteries were isolated, immersed in physiologic solutions at 1°C, transported to the laboratory, and cleaned. All experiments employed physiologic solutions aerated with 95% O$_2$ and 5% CO$_2$. The solutions contained 122.5 mM NaCl, 4.0 mM KCl, 1.2 mM MgSO$_4$7H$_2$O, 1.2 mM KHPO$_4$, 1.2 mM CaCl$_2$7H$_2$O, 17.5 mM NaHCO$_3$, 10 mM glucose, and 40 mM ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA). Potassium-rich solutions had 122.5 mM KCl and 17.5 mM KHCO$_3$ isosmotically substituted for NaCl and NaHCO$_3$. Calcium-deficient solutions had no CaCl$_2$. Concentrated HCl, NaOH, or KOH was used to adjust the pH of the solutions to 7.4. Solutions were freshly prepared; foil-wrapped containers were used to prevent light degradation.

The development of isometric tension was recorded with 0.125 M NaCl. At the concentrations used, no potassium solutions to 7.4. Solutions were prepared with deionized water, except for nifedipine, which was solubilized in dimethyl sulfoxide (DMSO) (57 μg nifedipine/ml DMSO), and [3-(3-hydroxy-1-octenyl)-7-oxabicyclo(2.2.1)hept-2-yl]-5-heptenoic acid) was a gift from Dr. D. Harris (Squibb Institute of Medical Research, Princeton, N.J.), Serotonin (5-hydroxytryptamine creatinine sulfate) was purchased from Sigma Chemical Co. (St. Louis, Mo.), $^{45}$Ca from ICN Radiochemicals (Costa Mesa, Calif.), and [3H]sorbitol from New England Nuclear (Boston, Mass.). Concentrated solutions of the agents were prepared with deionized water, except for nifedipine, which was solubilized in dimethyl sulfoxide (DMSO) (57 μg nifedipine/ml DMSO), and SQ-26,655, which was dissolved in ethanol and diluted with 0.125 M NaCl. At the concentrations used, no solvent induced changes in isometric tension. Solutions were freshly prepared; foil-wrapped containers served to prevent light degradation.

For the isometric tension experiments, arteries were cut into rings of uniform width (2.5 mm). Each ring was mounted vertically on two L-shaped stainless steel wire holders (0.4 mm o.d.) between a glass anchor and a Statham isometric transducer (Glen Burnie, Md.). The rings were maintained in 25–50 ml oxygenated physiologic solution (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 the arteries. The development of isometric tension was recorded on digital readout amplifiers and on Kipp and Zonen Model BD40 (Bohemia, N.Y.) and Gould Brush Model 816 (Glen Burnie, Md.) recorders.

For all $^{45}$Ca flux experiments, the arterial rings were opened into strips approximately 1.5 cm long. Each strip was quickly blotted on filter paper, weighed, and mounted on a stainless steel wire holder. After 3 hours' equilibration in a physiologic solution at 37°C, the strips were exposed to radioactive media (physiologic solution containing $^{45}$Ca [0.2 μCi/μmol Ca$^{2+}$], [3H]sorbitol [0.2 μCi/ml], and nonradioactive sorbitol [5.5×10$^{-6}$ M]). After incubation in the radioactive media at 37°C, the strips were blotted with a Kim-wipe tissue (Kimberly-Clark Corp., Roswell, Ga.), dipped momentarily in a calcium-deficient solution at 37°C, blotted again, and immersed in 50 ml ice-cold calcium-deficient solution containing 2 mM EGTA for 40 minutes. The strips were incubated overnight in 3 ml of 0.1N HNO$_3$ + 1% LaCl$_3$ at 25°C to extract retained $^{45}$Ca. Aliquots of the radioactive media, the calcium-deficient 2 mM EGTA solution, and the HNO$_3$ solution were added to 10 ml Scintiverse II scintillation cocktail (Fisher Scientific Co., Pittsburgh, Pa.) in 20-ml polyethylene vials, which were counted for $^{45}$Ca and $^3$H cpm in a Packard Tricarb liquid scintillation counter (Meriden, Conn.). Samples and blanks had equal volumes, so the raw counts required no quench correction.

In the $^{45}$Ca uptake experiments, the total $^{45}$Ca content of each strip (as micromoles per gram wet weight) was determined by adding the EGTA-extractable $^{45}$Ca content (i.e., that extracted from the strip into the EGTA solution) and the residual $^{45}$Ca content (i.e., that remaining in the HNO$_3$ solution). Each $^{45}$Ca content was calculated as ([$^{45}$Ca cpm/strip] x (μmol Ca$^{2+}$/ml media)] + ([3H cpm/strip] x (g wet wt/strip)), where cpm=increase in cpm above blank cpm. The volume of extracellular water was estimated with [3H] sorbitol; arteries were assumed to have a density of 1 ml/g. The total [3H]sorbitol space (as milliliters per gram) was calculated by adding the volumes of extracellular water in the EGTA and HNO$_3$ solutions. Each volume was calculated as (corrected $^3$H cpm/strip)/(1000 [3H cpm/ml media] x (g wet wt/strip)), where cpm=increase in cpm above blank cpm. The $^3$H cpm were corrected for $^{45}$Ca overlap on the $^3$H channel of the liquid scintillation counter as (total $^3$H cpm/strip) - ([3H cpm due to $^{45}$Ca/ml media] x ([3H cpm/strip] + ([3H cpm/ml media])], where cpm=increase in cpm above blank cpm. The $^{45}$Ca contents of the extracellular water in the EGTA and HNO$_3$ solutions (as micromoles per gram) were calculated as (1.2 μmol Ca$^{2+}$/ml media) x ([3H]sorbitol space). The extracellular bound $^{45}$Ca content for each strip equaled its EGTA-extractable $^{45}$Ca content minus the $^{45}$Ca content of extracellular water in the EGTA solution. The $^{45}$Ca uptake equaled the residual $^{45}$Ca content minus the $^{45}$Ca content of extracellular water in the HNO$_3$ solution.

For the $^{45}$Ca efflux experiments, after 3 hours' equilibration the strips were incubated in radioactive media at 37°C for 60 minutes. The strips were blotted and dipped as previously described and immersed in a series of 3-ml aliquots of calcium-deficient 2 mM EGTA solution at 37°C for 2 hours. Some strips...
were exposed to calcium-deficient 2 mM EGTA solution containing 10^{-7} M PGF_{2\alpha} or 10^{-3} M SQ-26,655 during the second hour. All strips were then incubated overnight in 3 ml of 0.1N HNO_{3} + 1% LaCl_{3} at 25°C to extract residual calcium. The data obtained were expressed as desaturation curves (which demonstrate the decline of strip ^{45}Ca content with time) and as curves showing the fractional rate of ^{45}Ca efflux (percentage of ^{45}Ca lost per minute).23

In all experiments, arterial rings or strips were selected at random to receive a drug or special treatment. Data are expressed as mean±SEM, with n≥6 rings or strips from at least five different animals for each variable. The null hypothesis was examined using analysis of variance and the Newman-Keuls multiple-range t test and was rejected at p<0.05. Calculations and statistical analyses were performed on microcomputers (Tandy TRS-80 Models III and IV, Fort Worth, Tex.) with BASIC or PHARM/PCS programs.29

**Results**

Both PGF_{2\alpha} and SQ-26,655 constricted arteries in a dose-related manner (Figure 1). Half-maximal constriction was produced by 2.3×10^{-6} M PGF_{2\alpha} and 1.3×10^{-9} M SQ-26,655. Maximal constriction was produced by 10^{-4} M PGF_{2\alpha} and 10^{-7} M SQ-26,655; the mean maximal tensions developed were 10.01±0.49 and 12.13±1.15 g, respectively.

When arterial rings were pretreated for 45 minutes with verapamil, nifedipine, or calcium-deficient solution, subsequent prostanooid-induced constriction was inhibited in a noncompetitive manner (Figure 1). Verapamil (10^{-5} M) or nifedipine (3.3×10^{-7} M) in concentrations sufficient to produce near-maximal inhibition of K^{+}-induced constriction of bovine cerebral arteries25 produced only half-maximal inhibition of prostanooid-induced constriction. Pretreatment with verapamil caused maximal PGF_{2\alpha} and SQ-26,655-induced constriction to be reduced to 45±7.2% and 42.6±5.8% of control, respectively. Pretreatment with nifedipine caused maximal PGF_{2\alpha} and SQ-26,655-induced constriction to be reduced to 49.9±7.3% and 46.6±7.6% of control, respectively.

Exposure to calcium-deficient solution produced near-maximal inhibition of prostanooid-induced constriction, as is the case for K^{+}- and serotonin-induced constriction of bovine cerebral arteries.25 The PGF_{2\alpha} and SQ-26,655-induced constrictions were reduced to 15.5±2.2% and 7.7±1.5% of control, respectively. Each prostanooid also produced slight constriction in calcium-deficient potassium-rich solution (Figure 1).

For the Ca^{2+} dose–response curve experiments, arteries were first pretreated with calcium-deficient solution for 45 minutes, with vasoconstrictors for 30 minutes, and with CaTs for an additional 30 minutes before Ca^{2+} was added. Verapamil and nifedipine had no effect on SQ-26,655-induced constriction in calcium-deficient solutions. These CaTs did produce slight (p<0.05) relaxation of arteries preconstricted with PGF_{2\alpha} in calcium-deficient solutions; control tension decreased 0.06±0.02 g, while tension decreased 0.18±0.02 g in the presence of verapamil and 0.18±0.04 g in the presence of nifedipine.

Calcium produced dose-dependent constriction when it was restored to calcium-deficient solutions containing PGF_{2\alpha} or SQ-26,655 (Figure 2). Verapamil and nifedipine inhibited the subsequent Ca^{2+}-induced vasoconstriction in a competitive manner. The CaTs caused the Ca^{2+} dose–response curve to be shifted to the right, although significant constriction still occurred at Ca^{2+} concentrations between 10^{-4} and 10^{-7} M.

To determine the effects of external Ca^{2+} concentration on ^{44}Ca uptake and contents, arterial strips were incubated for 60 minutes in radioactive media containing no EGTA and various concentrations of Ca^{2+} (from 15 to 3,000 μmol/l). The concentrations of Ca^{2+} in the incubating solutions were confirmed with atomic absorption spectrophotometry. The uptake of ^{44}Ca was dependent on the extracellular Ca^{2+} concentration (Figure 3); it ranged from 54±2 nmol/g in 15 μM Ca^{2+} to 130±7 nmol/g in 2,000 μM Ca^{2+}. External Ca^{2+} concentration had no effect on the total [H]sorbitol space.

Total and bound ^{44}Ca contents varied directly with the concentration of Ca^{2+} in the external bathing.
solutions (Figure 3). The use of a Scatchard plot of bound \(^{45}\text{Ca}\) content\(^{30-32}\) showed that bovine cerebral arteries possess two distinct (high- and low-affinity) calcium binding sites (Figure 4). The x-axis intercept yields an estimate of the number of high- or low-affinity sites on smooth muscle, while the affinity of each site can be expressed in terms of the apparent dissociation constant, \(K_d\), where \(K_d\) equals the x-axis intercept divided by the y-axis intercept.\(^{32}\) Based on the x-axis intercept values (4.63 and 0.47 µmol/g), low-affinity calcium binding sites outnumber high-affinity binding sites by approximately 10:1.

Both PGF\(_{2\alpha}\) and SQ-26,655 increased \(^{45}\text{Ca}\) uptake into bovine cerebral arteries during 5 minutes of \(^{45}\text{Ca}\) loading when the external \(\text{Ca}^{2+}\) concentration was 1.2 mmol/l (Figure 5); this \(^{45}\text{Ca}\) influx represents uptake from low-affinity sites on the arteries. For PGF\(_{2\alpha}\), \(^{45}\text{Ca}\) uptake increased from 69 to 108 nmol/g, and for SQ-26,655, from 78 to 141 nmol/g. Verapamil and nifedipine significantly blocked the increase in \(^{45}\text{Ca}\) uptake induced by PGF\(_{2\alpha}\) and SQ-26,655 (Figure 5); the resultant \(^{45}\text{Ca}\) uptake was not significantly greater than control. Verapamil, DMSO, nifedipine, PGF\(_{2\alpha}\), SQ-26,655, and serotonin had no significant effect on high-affinity \(^{45}\text{Ca}\) uptake. Only 144 mM K\(^+\) (isosmotically substituted for Na\(^+\) in the physiologic solution) increased high-affinity \(^{45}\text{Ca}\) uptake (<0.01); after 5 minutes K\(^+\)-induced \(^{45}\text{Ca}\) uptake was 25±3 nmol/g. Verapamil, nifedipine, DMSO, and the vasoconstrictors had no effect on total \(^{45}\text{Ca}\) content, total [\(^{3}\text{H}\)]sorbitol space, or bound \(^{45}\text{Ca}\) content after 5 minutes of tracer loading in radioactive media under high-affinity conditions (external \(\text{Ca}^{2+}\) concentration=45 µmol/l).

To determine if the agents had an effect on \(^{45}\text{Ca}\) influx from high-affinity binding sites, \(^{45}\text{Ca}\) influx was examined in radioactive media containing 45 µM \(\text{Ca}^{2+}\) (with no EGTA). Control high-affinity \(^{45}\text{Ca}\) uptake was 15±1 nmol/g after 5 minutes of \(^{45}\text{Ca}\) loading. Verapamil, DMSO, nifedipine, PGF\(_{2\alpha}\), SQ-26,655, and serotonin had no significant effect on high-affinity \(^{45}\text{Ca}\) uptake. Only 144 mM K\(^+\) (isosmotically substituted for Na\(^+\) in the physiologic solution) increased high-affinity \(^{45}\text{Ca}\) uptake (<0.01); after 5 minutes K\(^+\)-induced \(^{45}\text{Ca}\) uptake was 25±3 nmol/g. Verapamil, nifedipine, DMSO, and the vasoconstrictors had no effect on total \(^{45}\text{Ca}\) content, total [\(^{3}\text{H}\)]sorbitol space, or bound \(^{45}\text{Ca}\) content after 5 minutes of tracer loading in radioactive media under high-affinity conditions (external \(\text{Ca}^{2+}\) concentration=45 µmol/l).

In the \(^{45}\text{Ca}\) efflux studies, exposure to calcium-deficient 2 mM EGTA solution at 37°C produced a time-dependent reduction in \(^{45}\text{Ca}\) content.\(^{25}\) After the arteries were exposed to the calcium-deficient EGTA solution for 60 minutes, PGF\(_{2\alpha}\) (10\(^{-3}\) M) and SQ-26,655 (10\(^{-4}\) M) had no effect on the rate of \(^{45}\text{Ca}\) efflux.

**Discussion**

Vascular smooth muscle contraction depends on an increase in free cytoplasmic calcium.\(^{33,34}\) The \(\text{Ca}^{2+}\) for constriction is supplied from two main sources, the extracellular space and the sarcoplasmic reticulum.\(^{34}\)
FIGURE 4. Scatchard plot of $^{45}$Ca binding to bovine cerebral arteries at 37°C. The x-axis intercept estimates number of high-affinity (0.47 μmol/g) or low-affinity (4.63 μmol/g) Ca$^{2+}$ binding sites, while affinity can be expressed in terms of apparent dissociation constant, $K_d$. $K_d=x$-axis intercept/y-axis intercept.

Extracellular Ca$^{2+}$ can enter the cytoplasm through three different channels in the plasma membrane: voltage-dependent channels, receptor-operated channels, and leak-operated channels. In high concentrations, K$^+$ depolarizes the plasma membrane of cerebral arteries and promotes Ca$^{2+}$ influx through voltage-dependent channels. Receptor-mediated agents such as PGF$\alpha_2$ and TxA$_2$ promote Ca$^{2+}$ influx through receptor-operated channels independent of membrane depolarization or release Ca$^{2+}$ from internal stores such as the sarcoplasmic reticulum. Extracellular Ca$^{2+}$ can also passively cross the plasma membrane in the absence of excitation through leak-operated channels.

Organic CAs inhibit vascular constriction mainly by blocking Ca$^{2+}$ uptake through voltage-dependent channels, specifically the long-lasting or slow (L) calcium channels. The CAs also block Ca$^{2+}$ uptake through receptor-operated channels to a variable extent, depending on the blood vessel studied and the pharmacologic agent used to produce constriction. Drug competition and radioligand binding studies suggest that the four classes of CAs — the dihydropyridines (e.g., nifedipine, nimodipine, and nicardipine), the phenylalkylamines (e.g., verapamil), the benzothiazepines (e.g., diltiazem), and the piperazines (e.g., flunarizine and lidoflazine) — bind to distinct sites within or near the calcium channels. The present study demonstrates that PGF$\alpha_2$ and SQ-26,655 each constrict cerebral arteries in part by promoting the influx of extracellular Ca$^{2+}$ through Ca$^{2+}$-sensitive receptor-operated channels. When bovine middle cerebral arteries were pretreated with the CAs nifedipine and verapamil in concentrations sufficient to produce near-maximal inhibition of K$^+$-induced constriction, subsequent maximal PGF$\alpha_2$- or SQ-26,655-induced constriction was reduced by approximately 50% (Figure 1). When Ca$^{2+}$ was restored to calcium-deficient solutions containing PGF$\alpha_2$ or SQ-26,655 and either verapamil or nifedipine, Ca$^{2+}$-induced constriction was inhibited in a competitive manner (Figure 2). Both PGF$\alpha_2$ and SQ-26,655 increased $^{45}$Ca uptake, and the increases in uptake were blocked by verapamil and nifedipine (Figure 5). The bulk of extracellular calcium in vascular smooth muscle does not exist as free Ca$^{2+}$ but is bound by guest on May 29, 2017 http://stroke.ahajournals.org/ Downloaded from
bound to abundant anionic sites on the plasma membrane and the interstitium between muscle cells. This study demonstrates that cerebral arteries possess two distinct calcium binding sites, a high-affinity site and a low-affinity site (Figure 1). Peripheral vessels also have two distinct calcium binding sites. Such binding sites may be located within the glyocalyx or on the outer membrane surface of vascular smooth muscle. In bovine cerebral arteries, low-affinity binding sites outnumber high-affinity sites by approximately 10:1. Both PGF_{2alpha} and SQ-26,655 stimulate ⁴⁵Ca uptake into the arteries from low-affinity binding sites (Figure 5), as do K+ and serotonin; low-affinity sites predominate when the free extracellular Ca²⁺ concentration is 1.2 mM.

As is the case with human cerebral arteries, prostaglandin-induced constriction of bovine middle cerebral arteries is partially resistant to inhibition by CaTs. This CaT-resistant constriction is dependent on Ca²⁺ in the external media (Figures 1 and 2). The contractility studies show that PGF_{2alpha} and SQ-26,655 promote Ca²⁺ influx through CaT-insensitive channels, but the ⁴⁵Ca flux studies do not support this hypothesis. The ⁴⁵Ca uptake from low-affinity binding sites stimulated by PGF_{2alpha} and SQ-26,655 was completely blocked by verapamil and nifedipine (Figure 5). These prostanoids had no effect on Ca²⁺ uptake from high-affinity binding sites. Only K⁺ increased ⁴⁵Ca uptake from high-affinity binding sites, as is the case in peripheral vascular smooth muscle.

Both PGF_{2alpha} and SQ-26,655 appear to constrict cerebral arteries in part by releasing Ca²⁺ from a depletable intracellular storage site, most likely the sarcoplasmic reticulum. Each prostanoid produces some constriction in calcium-deficient solutions (Figure 1), indicative of internal Ca²⁺ release. The intracellular store is depleted when Ca²⁺ influx is blocked, so prostaglandin-induced constriction is inhibited to a greater extent by calcium withdrawal than by CaTs. The store is replenished by extracellular Ca²⁺ entering through leak-operated channels, so Ca²⁺ constricts arteries when it is restored to calcium-deficient solutions containing PGF_{2alpha} or SQ-26,655, even when CaTs are present (Figure 2). Exposure of arteries to calcium-deficient solutions inhibits arterial (leak-operated) ⁴⁵Ca uptake (Figure 3), while verapamil and nifedipine have no effect on basal ⁴⁵Ca uptake from either high- or low-affinity binding sites (Figure 5). Agents that release Ca²⁺ from intracellular stores would be expected to increase the rate of ⁴⁵Ca efflux, as is the case for two TxA₂ analogues acting on isolated rabbit aorta and human vessels. Neither PGF_{2alpha} nor SQ-26,655 increased ⁴⁵Ca efflux from bovine cerebral arteries, most likely because Ca²⁺ was depleted from the intracellular stores by exposure to calcium-deficient 2 mM EGTA solution for 60 minutes. Under similar conditions, PGF_{2alpha} also failed to stimulate ⁴⁵Ca efflux from bovine coronary arteries. An increase in ⁴⁵Ca efflux may be undetectable by the present methodology, or Ca²⁺ may translocate from one intracellular store to another.

In summary, PGF_{2alpha} and the stable TxA₂ mimetic SQ-26,655 potently constrict isolated bovine cerebral arteries. Constriction by either prostanoid was only partially blocked by the CaTs nifedipine and verapamil and was then further but not completely inhibited by calcium-deficient solutions. The results of these contractility studies are compatible with results reported by other investigators for cerebral arteries from humans and other species, indicating that bovine cerebral arteries are not atypical in their responses to PGF_{2alpha} stable TxA₂ analogues, and CaTs. Both PGF_{2alpha} and SQ-26,655 appear to constrict cerebral arteries by two mechanisms: by promoting Ca²⁺ uptake through receptor-operated channels in the plasma membrane and by releasing Ca²⁺ from depletable intracellular stores. The CaTs effectively block receptor-mediated Ca²⁺ uptake but not Ca²⁺ release; CaTs had no effect on basal or K⁺-stimulated ⁴⁵Ca efflux. Accordingly, CaTs may be limited in their ability to improve cerebral blood flow in cerebrovascular disorders such as stroke, postischemic hyperfusion after cardiac arrest, or cerebral vasospasm after subarachnoid hemorrhage if PGF_{2alpha} or TxA₂ is involved in the pathogenesis of these disorders.

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