Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels in corpus cavernosum smooth muscle: a novel mechanism for control of penile erection

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Karkanis, Tom, Ling DeYoung, Gerald B. Brock, and Stephen M. Sims. Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels in corpus cavernosum smooth muscle: a novel mechanism for control of penile erection. J Appl Physiol 94: 301–313, 2003. First published September 27, 2002; 10.1152/japplphysiol.00660.2002.—Little is known of the excitatory mechanisms that contribute to the tonic contraction of the corpus cavernosum smooth muscle in the flaccid state. We used patch-clamp electrophysiology to investigate a previously unidentified inward current in freshly isolated rat and human corporal myocytes. Phenylephrine (PE) contracted cells and activated whole cell currents. Outward current was identified as large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} current. The inward current elicited by PE was dependent on the Cl\textsuperscript{−} gradient and was inhibited by niflumic acid, indicative of a Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} (Cl\textsubscript{Ca}) current. Furthermore, spontaneous transient outward and inward currents (STOCs and STICs, respectively) were identified in both rat and human corporal myocytes and derived from large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} and Cl\textsubscript{Ca} channel activity. STICs and STOCs were inhibited by PE and A-23187, and combined 8-bromoadenosine cAMP and 8-bromoadenosine cGMP decreased their frequency. When studied in vivo, chloride channel blockers transiently increased intracavernosal pressure and prolonged nerve-evoked erections. This report reveals for the first time Cl\textsubscript{Ca} current in rat and human corpus cavernosum smooth muscle cells and demonstrates its key functional role in the regulation of penile erection.

The trabecular smooth muscle cells (SMCs) of the corpus cavernosum are the ultimate determinants of the erectile state of the penis (2). During the flaccid phase, these SMCs are partially contracted, thereby reducing the volume of the cavernosal spaces. Both \(\alpha_1\) and \(\alpha_2\)-adrenoreceptors are expressed in corpus cavernosum SMCs, and norepinephrine (NE) contributes to the tonic contraction by mediating Ca\textsuperscript{2+} release from intracellular stores (31, 40, 42).

However, evidence also exists for the role of Ca\textsuperscript{2+} entry in the tonic contraction of the corpus cavernosum SMCs. Indeed, in vitro studies have demonstrated myogenic tone in the absence of adrenergic signaling. Although myogenic contraction is unaffected by phenolamine, an \(\alpha\)-adrenergic antagonist (2, 10), this tonic contraction can be abolished by voltage-dependent Ca\textsuperscript{2+} channel (VDCC) blockers such as verapamil and nifedipine or by removal of extracellular Ca\textsuperscript{2+} (10, 14, 20). The dependence of myogenic tone on Ca\textsuperscript{2+} influx through VDCCs suggests that membrane potential plays an important role in corpus cavernosum SMC contraction and may be a key determinant for both the erectile and flaccid states.

In vascular myocytes, the membrane potential is an important determinant of Ca\textsuperscript{2+} influx and is regulated by hyperpolarizing K\textsuperscript{+} channel activity and depolarizing effects of either Cl\textsuperscript{−} or nonselective cation currents, ultimately controlling myogenic tone (4, 16, 17). Recent studies have demonstrated that local Ca\textsuperscript{2+} transients, or “sparks,” can activate these channels (32, 36, 47). Through transient activation of outward or inward currents, Ca\textsuperscript{2+} sparks are proposed to regulate the membrane potential and thereby Ca\textsuperscript{2+} influx through VDCCs (22). Although various K\textsuperscript{+} channels have been described in freshly dissociated corpus cavernosum smooth muscle (30, 44), a depolarizing and excitatory current is yet to be identified. Furthermore, the possible involvement of spontaneous transient currents in control of contraction of corpus cavernosum SMCs remains unexplored.

The process of erection requires the coordinated relaxation of the cavernosal SMCs and the vascular smooth muscle lining the arterial afferents to the penis. Although several vasoactive agents exert their effects on corporal SMCs, their mechanism of action is not completely understood. Nitric oxide (NO), released by both endothelial cells lining the trabecular spaces and nonadrenergic noncholinergic nerves, is the primary candidate for the corporal smooth muscle relaxation necessary for erection (7, 31). NO is believed to cause relaxation by activating K\textsuperscript{+} channels, thereby hyperpolarizing SMCs and closing VDCCs. This may occur either through direct interaction with K\textsuperscript{+} channels (6) or by activation of a cytosolic form of guanylyl cyclase and subsequent production of cGMP (3, 39),

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with evidence for the latter form of regulation on K+ channels in corpus cavernosum SMCs (29). However, recent reports have suggested alternate pathways by which NO can affect relaxation. For example, NO suppresses tonic Cl- currents in some SMCs, thus removing an excitatory conductance, which would favor hyperpolarization (16). Furthermore, NO affects Ca2+ uptake and release by intracellular stores, thereby altering intracellular Ca2+ concentration and Ca2+ signaling in vascular and airway muscles (12, 18, 24, 38).

Recent evidence indicates that NO signaling is necessary for the initiation of penile erection but may not be sufficient for its maintenance. For example, NO levels in the corpus cavernosum induced by cavernous nerve stimulation do not precisely match changes in cavernosal pressure in vivo. We demonstrate an excitatory conductance was present in these cells and to identify in freshly isolated corpus cavernosum SMCs. Furthermore, we demonstrate that ClCa channel blockers enhance and prolong the rise in pressure after cavernosal nerve stimulation, indicating that Cl current contributes to the regulation of intracavernosal pressure. This is the first demonstration of a depolarizing current in human and rat corpus cavernosal SMCs and provides new insight into the factors regulating the erectile process.

METHODS

Rat cell isolation. Male Sprague-Dawley rats of ~400 g were killed by injection of euthanyl (675 mg/kg ip). The penis was dissected out, and the glans and urethra were removed. The remaining tissue was cut into ~1 mm thick sections. These were placed in 2.5 ml of dissociation solution (see Solutions) plus the following: 0.8 mg/ml papain, 3.0 mg/ml bovine albumin, 0.5 mg/ml 1,4-dithiothreitol, 0.5 mg/ml Sigma blend collagenase type F, 1.0 mg/ml Sigma blend collagenase type F, 10 mM taurine, and 0.5 mM EDTA (pH 7.0). Tissues were either placed in a gently shaking water bath at 31°C for 120–180 min and dispersed by trituration with fire-polished Pasteur pipettes for immediate use or stored overnight at 4°C. The following day, tissues were warmed to room temperature for 120 min, then dispersed by trituration with fire-polished Pasteur pipettes.

Patch-clamp recording. The isolated cells were transferred to a 1-ml bath perfusion chamber and were allowed to settle and adhere before being perfused with bath solution at a rate of 1–3 ml/min. The chamber was mounted on the stage of a Nikon inverted microscope. For whole cell current recordings, the nystatin (250 μg/ml) perforated patch technique was used. All currents were recorded at room temperature (21–25°C) with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA), filtered at 1 kHz and sampled at 5 kHz by use of pCLAMP 6.0.4 software (Axon Instruments). Current and voltage were recorded by using a pulse code modulator (PCM-2; Medical Systems, Greenvile, NY) and displayed on a chart recorder (Gould RS-5200). Pipette resistance before seal formation was 2–3 MΩ. Whole cell current recordings was initiated when the access resistance became stabilized at <40 MΩ and up to 80% series resistance compensation could be applied. Liquid junction potentials of ~2 mv with CsCl pipette solution and ~10 mV with CsGlu pipette solution were corrected where appropriate. For single-channel recordings in the cell-attached patch configuration, cells were perfused with 135 mM KCl solution to chemically clamp the cell at 0 mV. Cells demonstrating spontaneous transient currents were identified by using Fetchan event-detection routines in pCLAMP with a current threshold of ~5 pA at ~40 mV (about three times the estimated single-channel large-conductance Ca2+-activated K+ channel amplitude at this potential), as reported previously (37). Only cells with spontaneous current events larger than this were included in the analysis. Test agents were applied by pressure ejection from glass micropipettes (Picospritzer II, General Valve, Fairfield, NJ) positioned 50–100 μm from cells.

Evaluation of intracavernosal pressure. A functional evaluation of the rat penile erection was determined by monitoring intracavernosal pressure (ICP) in live animals. Male, 20-wk-old Sprague-Dawley rats weighing ~450 g were anesthetized with 75 mg/kg ketamine and 5 mg/kg xylazine (ip). The lateral-prostatic space was dissected by utilizing a lower abdominal midline incision allowing isolation of the major pelvic ganglion and cavernous nerves. A stainless steel bipolar electrode with exposed tips ~2 mm apart was used to stimulate the cavernous nerve. Next, the penile crus were exposed by using a sagittal perineal incision. Microsurgery was facilitated by a Zeiss SR operating stereomicroscope. Penile pressure was recorded by insertion of a 23-gauge needle filled with heparinized saline (250 units/ml) into the right crura, connected by Tygon tubing to a pressure transducer (Baxter Health Care, Irving, CA). The amplified signal was digitized at 1.15 samples/s and stored on a Macintosh computer running LabVIEW 2 software (National Instruments, Austin, TX). Arterial pressure was recorded throughout the course of the experiment by cannulation of the carotid artery and use of the same pressure transducer and recording software. Corpoaral pressure changes were evoked with 0.2-ms pulses of 2 mA at 20 Hz for a 40 s duration by using the LabVIEW setup, parameters previously established to reliably evoke increases of intracavernosal pressure (1). To account for changes in magnitude and duration of the responses, we calculated the area under the pressure curves by using PRISM analysis software. A 30-gauge needle was inserted into the left crura for the administration of test com-
pounds. After completion of the experiments, the animals were killed by intraperitoneal injection of pentobarbital (200 mg/kg).

**Solutions.** The Krebs bicarbonate solution for retrieval of tissues consisted of (in mM) 116 NaCl, 5 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 2.2 NaH₂PO₄, 25 NaHCO₃, and 10 d-glucose, equilibrated with 5% CO₂-95% O₂ (pH 7.4). The dissociation solution used for cell dispersal contained (in mM) 135 KCl, 10 HEPES, 10 d-glucose, 1 CaCl₂, 1 MgCl₂, 10 taurine, and 0.25 EDTA (pH set to 7.0 with KOH). The Na⁺-HEPES bath solution used for perforated-patch recording contained (in mM) 130 NaCl, 5 KCl, 20 HEPES, 10 d-glucose, 2 CaCl₂, and 1 MgCl₂ (pH set to 7.4 with NaOH). For cell-attached patch recording, bath and electrode solution was composed of (in mM) 135 KCl, 20 HEPES, 10 d-glucose, 2 CaCl₂, and 1 MgCl₂ (pH set to 7.4 with KOH). The electrode solution used for perforated-patch recording contained (in mM) 140 KCl, 0.4 CaCl₂, 1 MgCl₂, 20 HEPES, and 1 EGTA (pH set to 7.2 with KOH). When K⁺ was replaced with Cs⁺, the electrode solution contained (in mM) 140 CsCl, 0.4 CaCl₂, 1 MgCl₂, 20 HEPES, and 1 EGTA (pH set to 7.2 with CsOH). When the Cl⁻ concentration was reduced by substitution with glutamate, the electrode solution was composed of (in mM) 40 CsCl, 100 glutamate, 0.4 CaCl₂, 1 MgCl₂, 20 HEPES, and 1 EGTA (pH set to 7.2 with CsOH).

**Chemicals.** All drugs and chemicals were obtained from Sigma Chemical (St. Louis, MO) or BDH (Toronto, Ontario, Canada) unless otherwise indicated. The 4-bromo A-23187 was purchased from Molecular Probes (Eugene, OR). Iberiotoxin was from Bachem.

**Statistical analysis.** Values are provided as means ± SE, with sample sizes (n) indicating the number of cells or animals studied. For patch-clamp experiments, only one patch was obtained per cell, and all findings were replicated on cells from multiple animals. Statistical comparisons were made by using the Student’s t-test or ANOVA when appropriate, with the Tukey-Kramer multiple-comparisons test for post hoc analysis. P < 0.05 was considered to indicate significance.

**RESULTS**

**Phenylephrine causes cell contraction and activates biphasic currents.** Isolated corpus cavernosum SMCs were spindle shaped and appeared phase bright under phase-contrast microscopy. When cells were stimulated with the α₁-adrenergic agonist PE (10 μM, applied at t = 5 s). Cell shortened to 30% of original length and returned to 73% after washout of PE. Of 39 cells tested, 36 contracted in response to PE. B: representative whole cell current traces from 3 different rat corpus cavernosum SMCs. PE, applied for time indicated by bars below current traces, evoked either a rapid and transient outward (top trace), biphasic (middle trace), or inward (bottom trace) current in cells held at −25 mV; 28 of 30 cells tested responded to PE with activation of current. Current saturation occurred in the middle trace, as indicated.

![Fig. 1. Phenylephrine (PE) causes cell contraction and activation of currents in rat corpus cavernosum smooth muscle cells (SMCs).](http://jap.physiology.org/)
Fig. 2. Identification of large-conductance Ca\(^{2+}\)-activated K\(^+\) channels in rat corpus cavernosum smooth muscle cells. Cells were bathed in solution containing 135 mM K\(^+\) to clamp the membrane potential at 0 mV. A: representative currents recorded at 100 mV. Single-channel events of \(-19\) pA were evident under control conditions. C, closed channels; O, open channels. B: patches were commanded from \(-50\) to 100 mV over 1.4 s with a ramp protocol to assess the current-voltage relationship of the single-channel currents. Three representative traces are superimposed and demonstrate increased channel activation at positive potentials, with no activity in any trace at patch potentials negative to 45 mV. C: current amplitude was determined at various potentials by using voltage step commands. Each point represents mean current \pm SE for 3 or 4 cells and yielded a conductance of 220 pS. D: representative current recording at 60 mV. Infrequent single-channel activity was recorded under basal conditions. PE (50 \(\mu\)M) was applied to the cell for 10 s and transiently increased channel activity (\(n = 3\)).

Fig. 3. PE activates a Ca\(^{2+}\)-activated Cl\(^-\) current in rat corpus cavernosum smooth muscle. A: representative whole cell current-voltage relationships demonstrating current activation by PE. K\(^+\) currents were blocked using 135 mM CsCl electrode solution. Cells were held at \(-60\) mV, and the potential was commanded from \(-100\) to 75 mV over 600 ms. The control trace demonstrates little current activation under basal conditions. After application of PE (10 \(\mu\)M), a large linear current was recorded that reversed slightly positive to 0 mV (as indicated by arrow; \(n = 5\)). B: Cl\(^-\) was reduced in the pipette solution to 43 mM by replacement with glutamate, resulting in a negative shift in the PE-activated current reversal potential (\(n = 4\)). C: Reversal potentials for PE-activated current in different recording conditions. When a 135 mM KCl electrode solution was used, Cl\(^-\) current reversal potential was \(11 \pm 7\) mV (KCl, \(n = 4\)). When K\(^+\) was replaced with Cs\(^+\), the reversal potential was recorded at \(5 \pm 3\) mV (CsCl, \(n = 5\)). On replacement of Cl\(^-\) with glutamate, the PE-activated current reversal potential shifted to \(-17 \pm 1\) mV (Cs glutamate (CsGlu), \(n = 4\)). \(*\) Significant difference of \(P < 0.05\). Off-line correction of liquid junction potentials was performed as stated in METHODS. D: Cl\(^-\) channel blocker ni flumic acid (NA) had no effect on basal currents (data not shown). When cells were challenged with PE in the presence of NA (NA + PE), no current activation was observed (\(n = 4\)). The PE-activated, NA-sensitive current was isolated by subtraction of the NA + PE trace in D from the PE trace in A, resulting in a linear current reversing at \(-7\) mV.

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because of the transient nature of the PE-activated currents, a voltage ramp protocol was employed to examine the current over a range of potentials. Cells were held at $-60 \text{ mV}$, and the potential was commanded from $-100 \text{ to } 75 \text{ mV}$ over 600 ms. When the electrode contained 140 mM KCl solution, the PE-activated inward current reversed at $17 \pm 1 \text{ mV}$ ($n = 4$). To isolate the PE-activated inward current, outward K$^+$ current was blocked by substitution of Cs$^+$ in the electrode solution. Under these conditions, the PE-activated current reversed at $5 \pm 1 \text{ mV}$ ($n = 5$). When the Cl$^-$ equilibrium potential was shifted from 0 to $-30 \text{ mV}$ by replacement of intracellular Cl$^-$ with glutamate, the PE-activated current reversal shifted to $-17 \pm 1 \text{ mV}$ (Fig. 3B, summarized in 3C; mean Cs glutamate reversal potential was significantly different from other conditions, $P < 0.01$ for each). Current reversal values were corrected for liquid junction potentials, whereas displayed traces were left unaltered. The 22-mV shift in reversal potential after Cl$^-$ substitution with glutamate suggested predominantly Cl$^-$ selectivity of the PE-activated current. The failure to shift by the predicted 30 mV may reflect the presence of an additional smaller component, such as a nonselective cation conductance, although this was not studied further here. This selectivity, in conjunction with the similar time course of activation as the PE-activated BKCa current, suggested that the inward current was a Cl$_{Ca}$ current. To further examine this current, we used the Cl$_{Ca}$ channel inhibitor niflumic acid (NA). Although it had no effect on basal currents recorded during voltage ramp commands, NA did block the effect of PE, confirming that activation of Cl$_{Ca}$ channels was responsible for the inward current (Fig. 3D).

Spontaneous transient currents in rat corpus cavernosum smooth muscle. In addition to agonist-activated macroscopic currents, spontaneous transient currents have been identified in various vascular and airway smooth muscles (23, 32). Spontaneous transient currents have not previously been identified in corpus cavernosum SMCs. During our investigation of the ion channels in the corpus cavernosum SMCs using the perforated patch technique, we observed spontaneous currents in 46% of the rat corpus cavernosum SMCs (84 of 182 cells studied). These were evident both as spontaneous transient outward currents (STOCs) and spontaneous transient inward currents (STICs). Figure 4 depicts a representative cell showing STOCs at a potential of $-25 \text{ mV}$, whereas only STICs were apparent at $-60 \text{ mV}$. At the intermediate potential of $-45 \text{ mV}$, biphasic events were evident, referred to as spontaneous transient outward then inward currents, or STOICs (47). The majority of these spontaneous currents were biphasic when recorded at an intermediate potential. However, the occasional solitary STOC or STIC was observed (middle trace, Fig. 4).

Pharmacological inhibition of STICs and STOCs. Previous analyses of STOCs have revealed that these events are due to the transient activation of K$^+$ chan-
nels. This was confirmed in the corpus cavernosum SMCs, in which application of tetraethylammonium caused complete inhibition of outward currents, whereas inward currents remained (5 mM, n = 3). After a 30-s washout of TEA, STOCs began to reappear. B: neither STOCs nor STICs were inhibited by 4-aminopyridine (4-AP; 5 mM, n = 6). C: STOCs were also abolished by iberiotoxin (IbTX, 100 nM), a selective inhibitor of the large-conductance Ca2+-activated K+ channel (n = 7). All currents were recorded at −25 mV.

Fig. 5. Pharmacological characterization of STOCs in rat corpus cavernosum smooth muscle. A: representative trace demonstrating reversible inhibition of STOCs with tetraethylammonium chloride (TEA), a nonselective K+ channel inhibitor. TEA caused rapid inhibition of all spontaneous outward currents, whereas inward currents remained (5 mM, n = 3). After a 30-s washout of TEA, STOCs began to reappear. B: neither STOCs nor STICs were inhibited by 4-aminopyridine (4-AP; 5 mM, n = 6). C: STOCs were also abolished by iberiotoxin (IbTX, 100 nM), a selective inhibitor of the large-conductance Ca2+-activated K+ channel (n = 7). All currents were recorded at −25 mV.

Fig. 6. Inhibition of STICs by Cl− channel blockers in rat corpus cavernosum smooth muscle cells. Both NA (A) and 4,4′-dinitrostilbene-2,2′-disulfonic acid (DNDS; B) selectively abolished STICs in rat cavernous smooth muscle (500 µM, n = 4 for each). Outward currents were still visible after inhibition of STICs. Currents were recorded at −45 mV from 2 independent cells.

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rents (Fig. 5C; 100 or 500 nM, n = 7), indicating that activation of these channels leads to STOCs.

Having identified a whole cell ClNa current in rat corpus cavernosum SMCs, we hypothesized that this channel was responsible for the STICs (and inward component of STOICs) seen in 24% of cells studied in the perforated-patch configuration. Indeed, both NA and 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS, a structurally distinct Cl- channel blocker) selectively inhibited the inward components of STOICs without affecting the outward currents (Fig. 6, A and B; 500 μM and n = 4 for each).

As demonstrated above, PE activated whole cell currents in corporal SMCs and elicited contraction. This is thought to involve the α1-adrenergic receptor, phospholipase C, and release of Ca2+ from intracellular stores by inositol 1,4,5-trisphosphate (31). In addition to activating macroscopic currents, PE also inhibited spontaneous transient current activity for cells exhibiting STOCs and STOICs (Fig. 7, A and B; n = 8). Moreover, application of the Ca2+ ionophore A-23187 (10 μM, n = 3) also inhibited spontaneous transient currents after an initial increase in both current amplitude and frequency. Together, these results indicate that manipulation of intracellular Ca2+ stores can inhibit spontaneous transient currents.

Spontaneous transient currents in human corpus cavernosum smooth muscle. We examined the properties of freshly isolated human corpus cavernosum SMCs to determine how they compared with those of the rat. Human tissue was retrieved after implant or corrective surgery. The small quantity of tissue, as well as the relatively rare occurrence of these surgeries, made single recordings more challenging. Through re-

![Fig. 7. Regulation of spontaneous currents by PE and A-23187 in rat corpus cavernosum smooth muscle. A: representative trace demonstrating the temporary inhibition of STOICs by PE (10 μM, n = 8). As demonstrated previously, PE activated an inward current, after which spontaneous currents were abolished. After a 60-s washout period, STOICs began to reappear. Current was recorded at −25 mV. B: in another cell, PE activated a transient outward current and also inhibited STOC activity. C: Ca2+ ionophore A-23187 briefly increased frequency and amplitude of spontaneous currents before causing an inhibition of all spontaneous events (10 μM, n = 3). Current saturation occurred in B and C.](http://jap.physiology.org/)

![Fig. 8. STICs, STOCs, and STOICs in human corpus cavernosum smooth muscle. Spontaneous currents were recorded from human corpus cavernosum smooth muscle cells. At −25 mV, only STOCs were recorded (top trace). At −45 mV, STOC amplitude decreased, and STOICs became apparent (middle trace). At −60 mV, only STIC activity was observed. Spontaneous currents were recorded in 4 of 10 cells studied, with 3 of 4 demonstrating STOICs and 1 of 4 only STOCs.](http://jap.physiology.org/)
Refinement of the dissociation enzyme concentrations, healthy cells, similar in appearance to those isolated from rat tissues, were obtained. Whole cell recording of isolated human corpus cavernosum SMCs revealed spontaneous currents comparable to those recorded from rat cells (Fig. 8). As in rat corpus cavernosum cells, human SMCs displayed STOC activity at $-25 \text{ mV}$. Membrane hyperpolarization resulted in a decrease in STOC amplitude and a concurrent increase in STIC amplitude, with only STICs apparent at $-60 \text{ mV}$ (4 of 10 cells). STOICs were also evident at intermediate potentials (Fig. 8, middle trace). When challenged with PE, human corpus cavernosum SMCs responded with the activation of a transient inward current at negative potentials and outward currents recorded at positive potentials (Fig. 9, A and B, $n = 4$). Although these currents were reminiscent of the BK$_{\text{Ca}}$ and Cl$_{\text{Ca}}$ currents activated by PE in rat cells, the limited supply of tissues precluded a more detailed analysis. In addition to activating whole cell currents, PE also inhibited STOCs when present in the human corporal SMCs (Fig. 9C). The inward current activated by PE and the spontaneous currents have not previously been described in human corpus cavernosum SMCs. Together, these results demonstrate a qualitative similarity to the rat SMCs investigated and indicate that rat corporal myocytes provide a valid model for human corpus cavernosum SMCs.

Cyclic nucleotides decrease spontaneous current frequency. Because cyclic nucleotides are important regulators of corpus cavernosum SMC contraction (31) and can regulate STOC frequency in vascular smooth muscle (37), we investigated the effects of cyclic nucleotides on the spontaneous currents. When cells demonstrating spontaneous current activity were perfused with combined 8-bromoadenosine 3',5'-cyclic monophosphate (8-BrcAMP) and 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP), membrane permeant forms of cAMP and cGMP, the frequency of the spontaneous events decreased (Fig. 10, A and B). The effect was transient, as demonstrated in Fig. 10A, and STOIC activity returned after washout. When each cyclic nucleotide analog was applied independently, the response was smaller and more variable, as shown for average responses (Fig. 10C). The frequency of spontaneous events was reduced to $73 \pm 24\%$ of basal in the presence of 2 mM 8-BrcAMP ($n = 4$) and $82 \pm 23\%$ with 2 mM 8-BrcGMP ($n = 3$). Neither change was significant, compared with the frequency decrease to $42 \pm 9\%$ in the presence of the combined cyclic nuclee-
otides (Fig. 10C; \( P < 0.05 \)), consistent with studies in human corpus cavernosum (26).

Previous studies have reported activation of K\(^{+}\) channels in smooth muscle by cyclic nucleotides (3). Although cAMP and cGMP decreased spontaneous current frequency, the magnitude of the outward and inward events was not consistently altered. To test the effects of the cyclic nucleotides over a broad range of potentials, we recorded whole cell currents by using the same voltage-ramp protocol described above. Combined 8-BrcAMP and 8-BrcGMP enhanced outward current at positive potentials without activation of the Cl\(_{Ca}\) current (Fig. 10D; 1 mM each; 11 of 12 cells tested). The selective increase in outward current suggests that cAMP and cGMP are affecting K\(^{+}\) channel activity without the elevation of Ca\(^{2+}\).

**Chloride channel blockers increase intracavernosal pressure in vivo.** To investigate a functional role for Cl\(_{Ca}\) channels in the corpus cavernosum, we used a rat model of erection in which ICP was monitored in vivo. This model allowed us to reproducibly induce erection by cavernosal nerve stimulation and quantify the amplitude and duration of the event. As a control, we first injected 50 \( \mu \)l of saline into the left crura at time zero, which caused no concomitant changes in ICP in the right crura. After a 5-min recovery period, the cavernosal nerve was stimulated, resulting in transient increases in ICP (Fig. 11A). Injection of 50 \( \mu \)l of NA into the left crura at 10 min caused a transient increase in ICP, in contrast to the equal volume of saline injected at the beginning of the trace. After a further recovery, nerve stimulation after injection of NA resulted in a greatly prolonged transient increase in ICP. To more clearly illustrate this, the first (control) and second (blocker) stimulation-induced pressure transients are superimposed in Fig. 11B, revealing the prolonged increase in ICP after NA. Similar elevation of ICP was observed after injection of the chloride channel blockers DNDS and 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (Fig. 11, A and B, middle and bottom). These blockers also transiently increased ICP on injection and also prolonged the nerve-evoked rise in pressure. However, DNDS did not prolong the nerve-evoked increase in ICP to the same extent as NA or SITS. Control studies confirmed that injection of saline alone had no effect on ICP and that repetitive stimulation was accompanied most often by a gradual decrease in the pressure change, opposite to what is observed in the presence of Cl\(^{-}\) channel blockers. In
addition, injection of the Cl\(^{-}\) channel blockers did not result in any change in mean arterial pressure.

To quantify the changes in ICP after injection of chloride channel blockers and stimulation, we calculated the area of the nerve-evoked ICP transients. As seen in Fig. 11A, injection of saline into the left crura of the rat produced no change in ICP. However, the area under the curve resulting from injection of NA, DNDS, and SITS all demonstrate significant increases, suggesting that blockade of chloride channels is sufficient to produce corpus cavernosum SMC relaxation and increased ICP (Fig. 12A, n = 3, P < 0.05). Moreover, when the area under the curve was compared for the stimulation-induced changes in ICP before and after injection of NA and SITS, significant differences were again observed (Fig. 12B). Values on stimulation increased from 40 ± 8 to 149 ± 23 cmH\(_2\)O·min after injection of NA (n = 3, P < 0.05). Similarly, stimulation-induced values increased from 19 ± 4 to 154 ± 43 cmH\(_2\)O·min on injection of SITS (n = 3, P < 0.05). This is the first evidence that Cl\(^{-}\) channel blockers can enhance nerve-stimulation-evoked rises in ICP.

DISCUSSION

In the present study, we provide evidence of an inward, excitatory current in freshly dissociated rat and human corpus cavernosum SMCs. This current is activated by the \(\alpha_1\)-adrenergic agonist PE and is voltage independent, selective for Cl\(^{-}\), and sensitive to NA, characteristics similar to the Cl\(_{Ca}\) currents described in vascular and airway smooth muscles (23, 28). Activation of the Cl\(^{-}\) current by PE is most likely mediated through transient release of Ca\(^{2+}\) from intracellular stores, given that PE also evokes reversible cell contraction and activation of BK\(_{Ca}\) channels. Furthermore, we demonstrate spontaneous transient currents in human and rat corporal myocytes, as well as their inhibition by PE and cyclic nucleotides. Lastly, through evaluation of ICP in vivo, we provide direct evidence that blockers of Cl\(_{Ca}\) channels enhance penile erection.

Excitation of corpus cavernosum SMCs with PE caused rapid contraction and activation of a biphasic current. Single-channel recording revealed the presence of a voltage-dependent channel that activated at positive potentials with a conductance of ~220 pS, similar to the BK\(_{Ca}\) channels of other SMCs (21, 25, 43). PE activated these channels despite their isolation under the patch pipette, indicating the involvement of a second messenger, most likely Ca\(^{2+}\). Both functional studies and single-cell patch-clamp experiments have previously demonstrated the role of the BK\(_{Ca}\) channel in contributing to the membrane potential of corpus
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Fig. 12. Cl⁻ channel inhibitors increase and prolong intracavernosal pressure. A: summary of the effects on ICP of injection of NA, DNDS, and SITS into the crura of the rat. Bars indicate means ± SE of integrated values of transient ICP increases on drug injection (50 μl, all injections). Saline resulted in no change in ICP, whereas all Cl⁻ channel blockers increased ICP. B: integrated values of ICP transients after cavernosal nerve stimulation. Both NA and SITS significantly increased ICP pressure area under the curve when injected 5 min before stimulation compared with control (con) stimulations after vehicle injection (P < 0.05).

cavernosum SMCs (19, 41), with recent evidence implicating KV currents as well (30). In this report, we confirm the presence of BKCa channels in corpus cavernosum SMCs. It is likely that KV channels were also active in these cells, because 4-aminopyridine decreased basal current at positive potentials to ~50%. However, these channels did not contribute to the formation of STOCs and so were not studied further here.

Both BKCa and KV channels hyperpolarize the membrane by allowing K⁺ efflux and thereby promote closing of VDCCs. However, it is through membrane depolarization and the opening of the Ca²⁺ channels that the SMCs of the corpus cavernosum are able to maintain tonic contraction during the flaccid state (10, 14, 20). In some vascular SMCs, membrane depolarization is achieved through the activation of Cl⁻ channels, because blockade of these channels causes membrane hyperpolarization and vessel dilation (28, 33). This finding supports the notion the Cl⁻ equilibrium potential is positive to the membrane potential in the resting state (28). Our results suggest that a similar mechanism may be active in the SMCs of the corpus cavernosum. Whole cell current recording revealed a ClCa channel in rat and possibly human cells, which was activated by PE and contributed to the formation of STICs. Furthermore, inhibition of the ClCa channels in vivo with multiple structurally distinct blockers resulted in a transient increase in ICP and a significantly prolonged nerve-evoked response, without affecting mean arterial pressure. These results suggest that ClCa channels are active before cavernosal nerve stimulation, while the corporal SMCs are contracted and the penis is in the flaccid state. Selective blockade of these channels in the corpus cavernosum removes a depolarizing mechanism, resulting in reduced Ca²⁺ influx and thereby SMC relaxation. This allows for increased blood flow into the trabecular space and results in a rise in ICP.

BKCa and ClCa channels require an increase in intracellular Ca²⁺ concentration to activate under physiological membrane potentials (9, 11, 28, 34). Although Ca²⁺ increases to the concentrations necessary for activation of these channels rarely occur throughout the entire cytosol of the cell, unitary Ca²⁺-release events from intracellular stores, or Ca²⁺ sparks, are believed to be of sufficient magnitude to activate localized Ca²⁺-dependent channels in the plasma membrane (5). These events are generally thought to be due to release of Ca²⁺ from ryanodine receptors, because low concentrations of ryanodine significantly enhance spark frequency and amplitude (35). Here we provide evidence for BKCa and ClCa channel activation at physiological membrane potentials, resulting in STOCs and STICs, respectively, in both rat and human corpus cavernosum SMCs. Although many spontaneous events were biphasic, resulting in STOICs, individual STICs and STOCs were also observed. Furthermore, release of Ca²⁺ from intracellular stores with PE inhibited spontaneous current activation, providing further evidence consistent with the involvement of Ca²⁺ sparks in corpus cavernosum. STOCs, elicited by Ca²⁺ sparks, have been identified in numerous SMCs and are known to play a role in the regulation of arterial diameter (8, 15, 37, 45). However, the function of STICs has not been as clearly elucidated. To date, STICs have primarily been observed in airway SMCs where they are thought to serve an antagonistic role to STOCs by providing a spark-induced depolarizing stimulus (27, 47). This would be consistent with the results of the present study, because Cl⁻ channel blockers specifically inhibited STICs during patch-clamp recording and also evoked a transient increase in ICP when injected into the corpus cavernosum. We propose that dynamic regulation of depolarizing STICs and hyperpolarizing STOCs may control Ca²⁺ influx through voltage-dependent channels and provide the tonic contraction of the corpus cavernosum during the flaccid state.

NO relaxes corpus cavernosum smooth muscle and mediates erection (7). In the present report, we show that cyclic nucleotides, known to be regulated by NO in
corporal myocytes, modulate the spontaneous current frequency. When combined, cAMP and cGMP decreased STOIC frequency by almost 60% in rat corporal myocytes. In comparison, cAMP and forskolin have been shown to significantly increase spark and STOC frequency in vascular SMCs, possibly through phosphorylation of ryanodine receptors or phospholamban (37, 46). Because those cells display no STICs, an increase in the frequency of sparks would result in greater hyperpolarization and therefore vessel dilation, although the physiological significance of this model remains uncertain (36). However, in the corpus cavernosum, where both hyperpolarizing and depolarizing currents are activated, an increase in spark frequency would not necessarily lead to cell relaxation. Reducing the frequency of spontaneous inward currents could represent a fundamental mechanism for relaxation of some vascular and airway muscles. By also reducing the frequency of STOCs, cyclic nucleotides may transfer control of the membrane potential to other, Ca2+-independent channels, such as the Kv channel, which has been shown to contribute to corpus cavernosum SMC membrane potential (30).

In conclusion, we provide the first demonstration of Cl\textsuperscript{ca} current in human and rat corpus cavernosum SMCs. This current represents a novel excitatory mechanism in corpus cavernosum SMCs. Its activation under basal conditions, seen as spontaneous transient currents, and the increase in ICP after blockade of Cl\textsuperscript{ca} channels suggest that it plays an essential role in the maintenance of myogenic tone and the regulation of penile erection. By blocking an excitatory influence, Cl\textsuperscript{ca} channel blockers promote vasodilatation, which is essential for erection. These studies reveal a new therapeutic target for treatment of erectile dysfunction.

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