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Reviving Cav1.2 as an attractive drug target to treat bladder dysfunction

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Abstract

Inhibition of bladder contraction with antimuscarinics is a common approach to treat bladder hyperactivity, and the L-type voltage-gated calcium channel α_{1C} (Cav1.2) is crucial for bladder contractility. Therefore, strategies aimed at inhibiting Cav1.2 appear warranted. However, multiple clinical trials that attempted to treat bladder overactivity with calcium channel blockers (CCBs) have been unsuccessful, creating an unsolved mystery. In contrast, cardiologists and epidemiologists have reported strong associations between CCB use and bladder hyperactivity, opposing expectations of urologists. Recent findings from our lab offer a potential explanation. We have demonstrated that ketamine which can cause cystitis, functions, like nifedipine, as a Cav1.2 antagonist. We also show that a Cav1.2 agonist which potentiates muscle contraction, rather than antagonizing it, can increase the volume of voids and reduce voiding frequency. This perspective will discuss in detail the unsuccessful urological trials of CCBs and the promise of Cav1.2 agonists as potential novel therapies for bladder dysfunctions.

Keywords

calcium channel blockers; overactive bladder; smooth muscle

1 | INTRODUCTION

Lower urinary tract symptoms (LUTS) constitute a highly prevalent group of urinary disorders affecting more than half of the population over 40 years of age.[1,2] Current drugs for LUTS include antimuscarinics and adrenergic β3 receptor agonists for pharmacological manipulation of bladder smooth muscle (BSM) contractility. The limited efficacy and high rates of side effects of these drugs lead to their frequent discontinuation by patients.[3–5] For example, the antimuscarinic drug Oxybutynin shows only marginal beneficial effects of 13%–25% over placebo for treatment of overactive bladder (OAB),[5] suggesting a clear unmet need to identify new molecular pathways and better targets for treating LUTS.

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AUTHOR CONTRIBUTIONS

Weiqun Yu conceived the idea, reviewed data, wrote the manuscript, reviewed and approved the manuscript.

DISCLOSURES

The author declares no competing interests.

The urinary bladder undergoes many cycles of filling and emptying every day, requiring coordinated contraction and relaxation of BSM. Accordingly, proper regulation of BSM contractility is critical for normal bladder function. The contraction stimulus that leads to voiding is initiated by the firing of parasympathetic motor neurons that release the neurotransmitters acetylcholine and ATP. Acetylcholine binds to muscarinic receptors M2 and M3, and ATP binds to purinergic receptor $P2X_1$.[6–8] Activation of these receptors depolarizes the BSM membrane potential, opening the L-type voltage-gated calcium channel, and causes calcium influx and BSM contraction. Conversely, activation of β3 adrenergic receptors by selective cate-cholaminergic drugs, and activation of the adenosine A2b receptor by the ATP metabolite adenosine relax BSM through inhibition of calcium channel-mediated calcium influx.[9,10] Therefore, modulation of calcium channel activity is crucial for BSM contraction and relaxation, and for normal bladder function.

2 | L-TYPE VOLTAGE-GATED Ca^{2+} CHANNELS

The long-lasting or the L-type voltage-gated calcium channel (LTCC) is a major subfamily of the ten voltage-gated calcium channels (VGCC). The LTCC subfamily constitutes four members, including Cav1.1, Cav1.2, Cav1.3, and Cav1.4. These LTCC channels are distributed widely across different types of cells. Cav1.1 is mainly expressed in skeletal muscle and some neuronal tissues, while Cav1.2 and Cav1.3 often show an overlapping expression pattern, and they are highly expressed in cardiac, smooth muscle, and neuronal tissues. Cav1.4 is expressed in the retinal cells for normal vision. LTCC channels are responsible for a variety of physiological functions such as the excitation-contraction coupling of muscle, and excitation of nerve and endocrine cells. LTCC channels are the only members of the VGCCs sensitive to 1,4-dihydropyridine (DHPs), and this unique pharmacological characteristic and their functional importance in many physiological processes make them attractive targets for drug discovery.[11]

3 | Cav1.2

Cav1.2 is essential for central nervous system function, cardiac and smooth muscle contractility, and neuroendocrine regulation.[12] Cav1.2 is expressed most abundantly in cardiac myocytes and BSM cells.[13] Cav1.2 channels are multi-subunit protein complexes composed of at least three subunits, designated α_{1C}, $\alpha_2\delta$, and β (Figure 1). The accessory subunits $\alpha_2\delta$ and β are noncovalently linked to the α_{1C} subunit. The α_{1C} subunit shapes the Ca^{2+} selective pore and contains the voltage sensor and the binding sites for most regulatory modulators and drugs. The α_{1C} subunit contains four repetitive transmembrane domains, and each transmembrane domain is composed of six transmembrane α-helices (S1–S6). Accessory subunits $\alpha_2\delta$ and β are involved in anchorage, trafficking, and regulatory functions.[12]

As an essential gene, Cav1.2 is important for channel-mediated Ca^{2+} signaling and plays a critical role in muscle contraction, hormone secretion, neuronal transmission, and gene expression. Mice globally null for Cav1.2 α_{1C} subunit are embryonic lethal, while tissue-specific deletion of Cav1.2 (cardiomyocytes, smooth muscle, and bladder) results in dramatic organ-level failure and death.[12] Gain-of-function mutations in two mutually

exclusive exons of the *Cav1.2* α_{1C} subunit *(CACNA1C)* gene cause Timothy syndrome (TS). TS patients exhibit a prolonged QT interval and lethal cardiac arrhythmias with an average life span of 2.5 years. Loss-of-function mutations of *Cav1.2* in patients with Brugada syndrome produce, in contrast, shortened QT interval predisposing to sudden cardiac death at an average age of 40.[14] In addition to these cardiac dysfunctions, patients with TS can also exhibit extensive abnormalities in different organs including central behavioral changes and intellectual deficits. However, whether these patients display urological symptoms has not been well reported.

4 | Cav1.2 TARGETING IN UROLOGY

Cav1.2 antagonists, including nifedipine, have been used for the management of hypertension since the 1970s because they inhibit vascular smooth muscle contractile force and reduce systemic vascular resistance.[15–18] As in vascular smooth muscle and cardiac muscle, Cav1.2 plays a critical role in bladder smooth muscle function. Total deletion of Cav1.2 specifically in mouse smooth muscle results in a dilated bladder with severely reduced micturition. The normal spontaneous contractile activity is absent in the bladders of these knockouts, and the muscle contraction force in response to carbachol or KCl is about 10-fold reduced, indicating a total loss of bladder function.[19,20] Consistent with these knockout animal studies, in vitro muscle strips studies have clearly indicated that CCBs such as nifedipine can inhibit BSM contraction. Nifedipine at a concentration of 0.01 μM starts to suppress the spontaneous contraction of rat BSM strips, which is completely inhibited at 3 μM. Nifedipine also dose-dependently inhibits up to 80% of carbachol, ATP, KCl, and electrical field stimulation-induced contraction in rodent BSM strips.[21] Likewise, nifedipine (0.1 $\mu g \cdot ml^{-1}$) completely blocks KCl, carbachol, and prostaglandin F2α-induced contraction in human BSM strips.[22] This excellent inhibitory effect of nifedipine on BSM contraction implies that Cav1.2 might be an attractive drug target to treat bladder dysfunction.

In fact, an initial trial of nifedipine for treatment of OAB patients took place more than 40 years ago.[22] In that trial, 19 patients with urgency and/or urgency incontinence were given 20–40 mg of nifedipine, which was taken orally 20 min before an acute in vivo urodynamic evaluation. To the investigators' surprise, only small and statistically non-significant changes were found in the urethral and bladder pressures. Bladder capacity also did not change (358 ml before, 365 ml after), however, there was a statistically significant increase in residual urine ($p < .05$), particularly in 7 of 19 patients who had residual urine of less than 25 ml before nifedipine and more than 50 ml after the drug. The average residual urine increased from 11 ml to 156 ml ($p < .01$).[22] The authors thus concluded that nifedipine lacks effectiveness to treat patients with urgency and/or urgency incontinence. Since then multiple additional CCBs have also been tested by urologists as treatments for patients with bladder hyperactivity and/or urinary incontinence, but mostly without therapeutic success.[23–26] In a study of 30 patients suffering from bladder instability and incontinence, urodynamics was studied before and 30 min after 10 or 20 mg of nifedipine oral intake. Interestingly, although statistically insignificant, nifedipine decreased bladder compliance (from 48.4 ml/cm H_2O to 40.6 ml/cm H_2O). Consistent with the decreased compliance, the volume required for spontaneous detrusor contractions significantly decreased in nifedipine-treated patients (from 250.4 to 208.6 ml, $p = .0008$). These findings demonstrated that

nifedipine did not increase bladder volume and did not suppress bladder instability.[23] Nimodipine, another LTCC blocker, was evaluated in a 2002 report in which 76 urge incontinence patients were treated with 30 mg nimodipine twice daily. The treatment was a randomized, double-blinded, and placebo-controlled crossover trial. However, nimodipine did not result in a significant improvement in the number of incontinent episodes ($p = .62$), amount of urine leakage ($p = .94$), or symptomatic urinary urgency ($p = .84$). Therefore, the authors concluded that nimodipine was not effective for the treatment of urge incontinence.[24] Verapamil, another LTCC blocker was tested by intravesical instillation in 22 patients with bladder hyperreflexia or idiopathic detrusor instability. The results indicated that verapamil could significantly increase the bladder capacity in those patients with hyperreflexia (from 236 to 395 ml), but not in patients with detrusor instability. However, no significant differences were found in other urodynamic parameters analyzed.[25] Verapamil is also an effective alpha-adrenoceptor and muscarinic receptor antagonist.[27] Therefore, the mechanism behind the limited effect of verapamil on patients with hyperreflexia is questionable. In contrast, oral administration of the LTCC blocker diltiazem had been reported to reduce the frequency of diurnal and nocturnal micturition and incontinence episodes in patients with detrusor hyperactivity.[28] However, there are no LTCC blockers approved for patients with bladder dysfunctions, and it is the general consensus that LTCC antagonists lack clinical effectiveness in these patients. Therefore, the failure of pharmacological inhibition of BSM contraction by CCBs to benefit patients with bladder overactivity has remained a clinical disappointment and a challenge to our understanding of bladder physiology.

5 | DATA ON Cav1.2 ANTAGONISTS FROM CARDIAC AND EPIDEMIOLOGIC STUDIES

In contrast to the lack of effects, or perhaps more correctly, the lack of targeted effects in the aforementioned urological studies, cardiologists Williams et al. reported in *The Lancet* in 1986 that nine patients on nifedipine (10–20 mg, 3 times/day) experienced nocturia (4 times/night), which ceased or improved when the drug was discontinued.[29] In another survey reported in *The Lancet* in 1988, 12 (7 men and 5 women) of 157 patients treated with nifedipine (30–60 mg/day) developed nocturia (4 times/night), which again ceased after stopping nifedipine.[30]

In the past decade, associations between CCB use and LUTS have emerged in large-scale clinical studies. A study in 206 Japanese male hypertension patients receiving CCBs experienced significantly higher LUTS symptoms. The International Prostate Symptom Score (IPSS) in these patients was significantly higher than in hypertension patients not receiving CCB treatments (19.6 vs. 16.2, $p < .05$), and they tended also to develop statistically higher frequency and greater severity of symptoms such as intermittency ($p < .01$), urgency ($p < .05$), and nocturia ($p < .001$) than did men not on CCB.[31] In a survey of 5503 Boston residents, the prevalence of LUTS among users of common anti-hypertension drugs was compared with non-users. CCB was again associated with increased prevalence of nocturia ($p < .01$), voiding symptoms ($p = .04$), and storage symptoms ($p = .09$) particularly in women.[32] An Australian cross-sectional study of 278 inpatients demonstrated a higher

prevalence and greater severity of LUTS in both sexes among CCB users than non-users ($p < .001$). The average IPSS score for CCB users was significantly higher than non-users (15.2 vs. 9.3, $p < .001$) in both males and females. The symptoms of CCB users experienced were more severe than non-users, including incomplete emptying ($p = .005$), frequency ($p < .001$), intermittency ($p < .001$), urgency ($p < .001$), weak stream ($p = .001$), and nocturia ($p < .001$). Therefore, CCB users were more likely to receive medical treatment for LUTS such as OAB (22.4% vs. 9.3%, $p = .003$), and undergo urogenital surgeries (16.5% vs. 7.8%, $p = .029$).[33] Indeed, manufacturers of Cav1.2 antagonists (CCBs) have included lower urinary tract symptoms (polyuria, dysuria, hematuria, and nocturia) among the listed side effects in their drugs' product information sheets (https://www.pfizermedicalinformation.com/en-us/procardia-xl?section=adverse-reactions).

The association of CCB use with intermittency, frequency, urgency, and nocturia, which is in direct contrast to the long-held clinical urologic assumption that inhibition of BSM contraction increases bladder volume and reduces voiding frequency. This assumption has been based on the beneficial effects in a subset of OAB patients of antimuscarinic inhibition of BSM contraction. It has been assumed, therefore, that inhibition of BSM contraction by CCBs should cause the same urological effects as antimuscarinic drugs. However, almost 50 years' worth of clinical experience with, and trials of CCBs, confirm the opposite.

6 | PHARMACOPHYSIOLOGY OF NIFEDIPINE, BAY k8644, AND KETAMINE

As noted earlier, nifedipine was initially developed for treating hypertension, because it inhibits vascular smooth muscle contractility by blocking Cav1.2, and thus reduces the vascular resistance for blood flow.[34–36] In order to understand the unsuccessful application of nifedipine and other CCBs for treating voiding dysfunctions like over-activity, it is helpful to review the cardiovascular effects of nifedipine and its side effects, which might inform our understanding of its role in bladder physiology.

Cav1.2 is highly expressed in cardiomyocytes, in addition to its expression in smooth muscle cells.[13] Thus, Cav1.2 also reduces the contractile force of the cardiac muscle to contribute to blood pressure reduction. However, in addition to reducing blood pressure, nifedipine commonly induces tachycardia, a fast, irregular, or racing heartbeat.[36–38] This on-target effect is caused by the combination of decreased left ventricular ejection fraction and increased vascular volume (due to rapid inhibition of both cardiac and smooth muscle contractility). In 2019, the European Sudden Cardiac Arrest Network (ESCAPE-NET) reported that common anti-hypertensive drugs such as nifedipine and amlodipine are associated with an increased risk of sudden cardiac arrest due to ventricular tachycardia/ventricular fibrillation.[39] Tachycardia in nifedipine users often resolves relatively quickly, because sinus rhythm is also controlled by the sinoatrial node pacemaker activity and its peripheral nervous inputs. However, in a fraction of patients on nifedipine, increased heart rate persists throughout treatment.

In contrast, the Cav1.2 agonist Bay k8644, which strongly potentiates cardiac contractility, has shown the opposite effect. Administration of a single dose of Bay k8644 transiently elevates rat arterial pressure up to 20%, which then returns to baseline. Bay

k8644 simultaneously causes a dose-dependent decrease in heart rate of up to 40% (Bradycardia).[40,41]

If the above observations of drug action in the heart apply also to the bladder, then we should expect nifedipine to decrease bladder contractile force and pressure (as with blood pressure) and increase voiding frequency and/or rate (as with heart rate). Numerous studies confirm that CCBs like nifedipine dose-dependently inhibit both human and animal BSM contraction in vitro.[22,42–44] Nifedipine at sub-micromolar level has been shown to effectively inhibit most of the carbachol or KCl-induced human BSM strip contraction.[22,45] Likewise, nifedipine at a similar dosage has shown the same effects on BSM strips from mouse, rat, rabbit, and pig.[43,46–50] These observations are consistent with well-known clinical side effects of nifedipine, including dizziness, edema or swelling in legs, constipation, flushing, and nausea, and indicate that nifedipine at clinical dosage has extensive effects on smooth muscle contractility. Therefore, nifedipine likely decreases in vivo bladder contraction pressure and increases voiding frequency, based on cardiologists' clinical experience and the larger clinical studies reviewed above, and based on the ability of nifedipine effectively to inhibit contraction of BSM from humans and multiple experimental animal species described above.

Prompted by the rising prevalence of ketamine abuse-related cystitis, a painful bladder syndrome with urinary urgency, frequency, and incontinence, we recently demonstrated that ketamine functions as a novel Cav1.2 antagonist.[46] Intrigued by this discovery, we looked further into the effects on in vivo bladder function of the Cav1.2 antagonist nifedipine and the Cav1.2 agonist Bay k8644. We, therefore, infused nifedipine and/or Bay k8644 into the bladder lumen during cystometrogram (CMG) studies, in an attempt to restrict drug effects to the bladder while minimizing systemic effects. This approach allowed detailed observation of the full-time course of drug effects immediately after its application to the same animal, increasing data reliability. Plasma nifedipine concentration in human patients after a single oral dose can reach up to ~300–400 ng/ml (~1 μM),[51] and myography revealed significant inhibition of mouse BSM contraction at this concentration.[46] Nifedipine exhibits a dose-dependent (0.1–100 μM) effect on bladder function in vivo during our CMG studies (Figure 2).[46] As expected, nifedipine dose-dependently inhibited bladder peak pressure, while concomitantly shortening voiding interval, with no instances of increased voiding interval at any tested concentration of nifedipine. These data are entirely consistent with previous cardiologists' reports on CCBs, and could partially explain why previous trials of CCBs including nifedipine to treat voiding dysfunction were unsuccessful.

By contrast, co-administration of Bay k8644 completely corrected the nifedipine-induced abnormality, increasing peak pressure and elongating the voiding interval (Figure 2). We and others have now shown that the Cav1.2 agonist Bay k8644 potentiates BSM contraction force significantly in vitro.[43,46,52,53] In contrast to nifedipine's inhibition on bladder muscle strips from different species, Bay K8644 at sub-micromolar level potentiated contraction force up to 2–3 fold on bladder muscle strips from human, mouse, rat, and rabbit. The ability of Bay k8644 to increase voiding pressure and to reduce the voiding frequency in vivo, although counter to current urologic dogma, is very consistent with Bay k8644's ability to increase cardiac output and reduce rodent heart rate.[40,41]

7 | POTENTIAL MECHANISMS

It is a well-accepted assumption in urology that inhibition of BSM contraction will increase bladder volume and therefore decrease voiding frequency, however, our observation seems counter-intuitive. As mentioned above, nifedipine can cause tachycardia due to decreased left ventricular ejection fraction. Nifedipine can also cause swelling in the legs and constipation likely due to impaired smooth muscle contraction force. Early trials on human patients with urgency also noted that oral intake of nifedipine (20–40 mg) caused an increase in residual urine.[22] It is thus plausible that nifedipine inhibition of BSM causes impaired voiding efficiency and thereby increases residual volume, and indeed increased residual volume has been reported in patients treated with nifedipine.[22] The increased residual volume will further be likely to increase voiding frequency because of reduced space available for the urine generated by kidneys. This hypothesis could be tested by performing a CMG study. During a normal CMG cycle in a mouse, immediately after the return of intravesical pressure to baseline following a normal void, the residual volume in the mouse bladder can be measured by switching the pump setting from infusion to withdrawal until intravesical pressure reaches ~0 cm H_2O, followed by registration of a steep pressure drop due to a vacuum effect (Figure 3). This withdrawal time can serve as an estimate of residual bladder volume. If our hypothesis is true, then we expect to observe that intravesical infusion of nifedipine with PBS will significantly increase withdrawal time (residual volume) as compared to control, which will confirm that inhibition of BSM by nifedipine causes significantly reduced voiding efficiency (defined as the degree of emptying from a full bladder, such that increases in residual volume imply reduced voiding efficiency). In contrast, we can also test whether the infusion of Bay k8644 can increase voiding efficiency by reducing residual volume (decreased withdrawal time). The conclusion from these studies will emphasize that strong BSM contraction force is required to maintain normal bladder function.

Why, then, does inhibition of muscarinic receptors but not of Cav1.2 benefit some LUTS patients? It may be that muscarinic receptors mainly signal initiation of BSM contraction (depolarizing the membrane to activate Cav1.2, such that inhibition of muscarinic signaling will reduce the input of the activating signaling), whereas Cav1.2 signaling is more directly responsible for the generation of BSM contraction force. Deletion of Cav1.2 results in total loss of BSM contraction and bladder function.[19,20] In contrast, muscarinic M2 and M3 double knockout mice are viable and grossly healthy. Males (but not females) exhibit significant urinary retention, and reduced bladder contraction force was characterized in male, but not in female M2/M3 double knockouts.[54]

Purinergic signaling, another important pathway mediating bladder contraction, may compensate blockade of muscarinic signaling. In a rodent study, chronic anticholinergic administration induced a shift from muscarinic to purinergic transmission in the bladder wall.[55] Thus, these data infer that muscarinic blockade at clinical dosage may mediate fine adjustment of bladder muscle tone without significantly altering bladder contraction force, whereas Cav1.2 inhibition will decrease smooth muscle contractile force and thus impact gross bladder function.[46] Note that anticholinergics also cause urine retention in some

patients, and further studies will be required to better understand the mechanisms behind these bladder responses.

8 | POTENTIAL CLINICAL TRANSLATION FOR LUTS

As mentioned above, intravesical administration of Bay k8644 can immediately reverse nifedipine- or ketamine-induced voiding frequency and abnormality (Figure 2).[46] These observations suggest that instead of Cav1.2 antagonists, Cav1.2 agonists such as Bay K8644 could be used clinically to treat LUTS. Indeed, we have observed that intraperitoneal injection of Bay K8644 significantly increased void volume, suggesting increased bladder capacity and/or voiding efficiency upon Cav1.2 agonist administration.[46] These data are contrary to current urologic expectations and could justify the revival of Cav1.2 as an attractive drug target for LUTS. Although the possible benefit to the urinary system of long-term administration of Cav1.2 agonists remains unknown, our CMG studies of intravesical Bay k8644 infusion showed persistently elongated voiding intervals (Figure 2), and our in vivo intraperitoneal injection of Bay k8644 also exhibited enlarged voids in studies of longer duration,[46] suggesting the possibility of developing a treatment schedule and dosing regimen allowing further study of Bay k8644 for chronic urinary system problems.

Unlike the readily available and widely used CCBs, no Cav1.2 agonists are approved for clinical application. The agonists Bay K8644 and FPL 64176 are widely used in laboratory studies, but due to wide tissue expression of Cav1.2 and poor isoform selectivity of these agonists, off-target side effects remain important concerns that constitute principal challenges to clinical use. For example, treatment of mice with high doses of Cav1.2 agonist Bay k8644 induced a depressive-like phenotype with self-injury behavior (neuronal effect).[56,57] In our studies, Bay k8644 administration also induced diuresis, likely reflecting increased cardiovascular output and/or increased renal filtration.[46] A possible approach to this problem may be to develop isoform-selective Cav1.2 agonists for bladder smooth muscle. In a study on CCB-induced LUTS, patients treated with vascular selective felodipine/lercanidipine did not develop a higher rate of LUTS compared to control,[33] suggesting a potentially significant difference between the isoforms of Cav1.2 in BSM and vascular smooth muscle. Indeed, >20 human *Cav1.2* gene splice variants have already been identified, and Cav1.2 isoform-selective drugs were long ago developed for hypertension.[58,59] Interestingly, different regulatory promoters have been found controlling the expression of genes in vascular and visceral smooth muscle cells,[60] and heterogeneity of tissue-specific gene expression has been found between visceral and vascular smooth muscle cells.[61] Thus, a careful study of BSM *Cav1.2* variants might foster the development of isoform-selective Cav1.2 agonists for LUTS.

9 | CONCLUSIONS AND FUTURE DIRECTIONS

Cav1.2 is a protein that is critical for mediating smooth muscle contraction and bladder function. Discoveries further elucidating the actions of Cav1.2 agonists and antagonists on urinary bladder function should provide promising avenues for the development of therapies for LUTS, which may eventually revive Cav1.2 as an attractive drug target for these problems. However, many unanswered questions need to be addressed in order

to achieve this goal. For example: (1). a clear mechanism behind Cav1.2 agonist and antagonist-induced bladder functional changes needs to be established; (2). a comparative study defining BSM *Cav1.2* isoform and isoforms in other tissues, including neurons, vascular smooth muscle, cardiomyocytes, etc. needs to be performed; (3). a comparative study of structure and function relationships between BSM Cav1.2 isoform and isoforms in other tissues needs to be performed; (4). based on knowledge accumulated from questions 1–3, modifications to, and selection of novel chemical compounds should be undertaken and may lead to a drug candidate(s) targeting Cav1.2 activation that is suitable for the treatment of LUTS; (5). with the rapid evolution of biotechnology, innovative therapeutic approaches targeting Cav1.2 may also be developed, such as targeted delivery systems. In conclusion, with the potential to revive Cav1.2 as an attractive drug target for LUTS treatment, a must-have prerequisite is a complete understanding of the underlying mechanisms by which CCBs failed and by which Cav1.2 activation appears to represent an effective drug strategy for LUTS.

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Abbreviations:

BSM	bladder smooth muscle
CCBs	calcium channel blockers
CMG	cystometrogram
DHPs	1,4-dihydropyridine
IPSS	International Prostate Symptom Score
LUTS	lower urinary tract symptoms
LTCC	L-type voltage-gated calcium channel
OAB	overactive bladder
TS	timothy syndrome
VGCC	voltage-gated calcium channels

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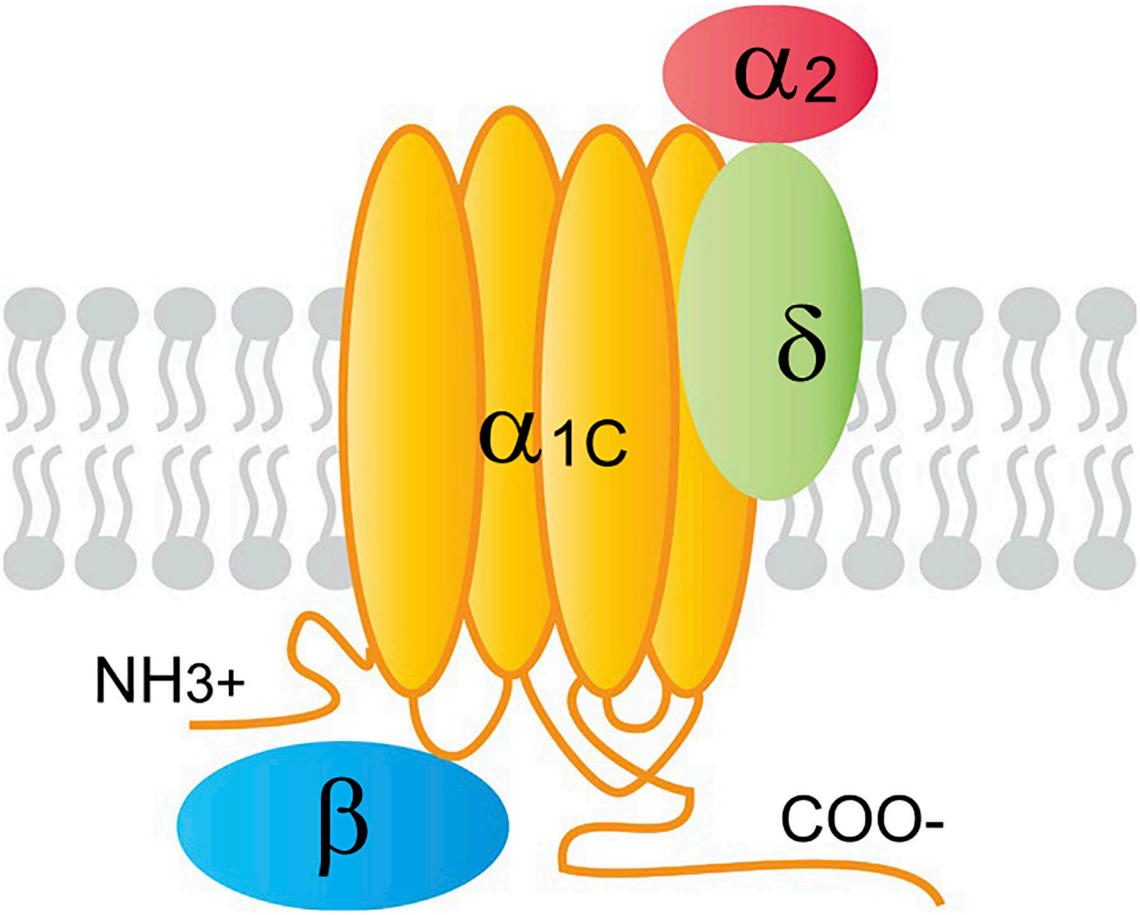

FIGURE 1.

Schematic of Cav1.2 calcium channel. Cav1.2 channels are multi-subunit protein complexes composed of at least three subunits, designated α_{1C}, $\alpha_2\delta$, and β. The Accessory subunits $\alpha_2\delta$, and β are noncovalently linked to the α_{1C} unit. The α_{1C} subunit shapes the Ca^{2+} selective pore and contains the voltage sensor and the binding sites for most regulatory modulators and drugs. The α_{1C} subunit contains 4 repetitive transmembrane domains, and each transmembrane domain is composed of six transmembrane α-helices (S1–S6). Accessory subunits $\alpha_2\delta$, and β are involved in anchorage, trafficking, and regulatory functions

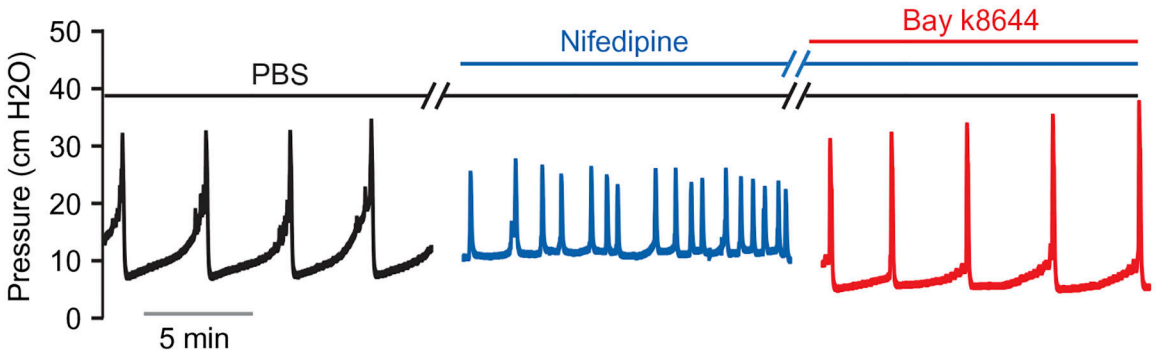

FIGURE 2.

Nifedipine-induced voiding frequency and diminished peak pressure are reversed by Bay k8644. Representative CMG traces are shown, at left is normal control superfused with PBS. Nifedipine (10 μM) infusion into the bladder lumen (middle) decreases the voiding interval and peak pressure. Subsequent intravesical infusion of Bay k8644 (200 nM) in the continued presence of nifedipine restored the voiding interval and peak pressure to normal values (right panel). Modified from Ref. [46]

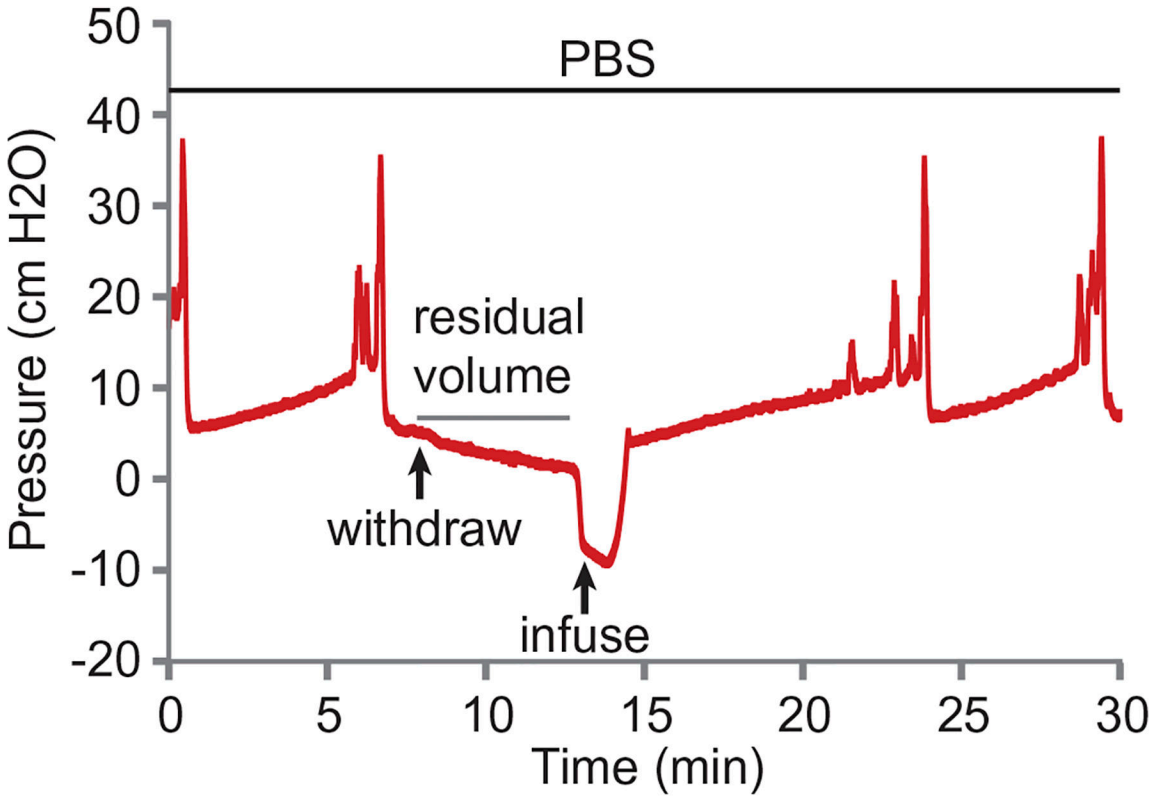

FIGURE 3.

Estimation of post-void residual volume by CMG. PBS infusion into the bladder during CMG induced repetitive normal filling and voiding pressure cycles. To estimate the post-void residual volume, we reversed the pumping direction from infusion to withdrawal, as indicated by the slow decrease in pressure until, upon reaching ~0 cm H_2O, pressure drops sharply due to the vacuum effect of the collapsed bladder, indicating a completely empty bladder/urinary lumen. Switching back to infusion restored normal filling and voiding cycles as shown. The time required to completely empty the bladder is the estimate of residual volume (from withdraw to infuse)