Current Therapeutic Research 99 (2023) 100717

Contents lists available at ScienceDirect

Current Therapeutic Research

journal homepage: www.elsevier.com/locate/curtheres

Inhibition of Contractility of Isolated Caprine Detrusor by the Calcium Channel Blocker Cilnidipine and Reversal by Calcium Channel Openers

Steffi A. Maria, MD, Aniket Kumar, PhD, Premila M. Wilfred, MD, Margaret Shanthi, MD, Jacob Peedicayil, MD*

Department of Pharmacology & Clinical Pharmacology, Christian Medical College, Vellore, India

ARTICLE INFO

Article history:
Received 11 July 2023
Accepted 26 September 2023

Key words:
Calcium channel
cilnidipine
contractility
detrusor
urinary bladder

ABSTRACT

Background: Cilnidipine is a fourth-generation calcium channel blocker that is clinically used to treat hypertension. It is a dihydropyridine that blocks L- and N-type calcium channels. The inhibitory effect of cilnidipine on isolated detrusor muscle contractility has not been studied. This study investigated the inhibitory effect of cilnidipine on isolated caprine (goat) detrusor muscle contractility and the reversal of the inhibition by calcium channel openers.

Methods: Fourteen caprine detrusor strips were made to contract using 80 mM potassium chloride before and after addition of three concentrations (20, 40, and 60 μM) of cilnidipine. Two reversal agents, the L-type calcium channel opener FPL64716, and the N-type calcium channel opener GV-58, were investigated for their ability to reverse the inhibitory effect of 40 μM cilnidipine on potassium chloride-induced detrusor contractility.

Results: Cilnidipine caused a dose-dependent and statistically significant inhibition of detrusor contractility at all concentrations of cilnidipine used (20, 40, and 60 μM). The inhibitory effect of 40 μM cilnidipine on detrusor contractility was significantly reversed by the addition of FPL64716 and GV-58.

Conclusions: Cilnidipine inhibits the contractility of the isolated detrusor by blocking L- and N-type calcium channels. Cilnidipine could be evaluated for treating clinical conditions requiring relaxation of the detrusor such as overactive bladder.

Introduction

Cilnidipine is a relatively new fourth-generation dihyropyridine calcium channel blocker (CCB) that is used clinically for the treatment of hypertension.[1,2] Unlike the older dihydropyridine CCBs such as nifedipine, which block mainly the muscle L-type voltage-gated calcium channels (VGCC), cilnidipine blocks both L- and neuronal N-type VGCC.[1,2] L-type VGCC in smooth muscle myocytes are also termed Cav1.2 channels and N-type VGCC at nerve terminals are also termed Cav2.2 channels.[3,4] By blocking N-type VGCC, cilnidipine inhibits sympathetic nerve terminal function, thereby exerting a sympatholytic effect.[1–4] This is believed to contribute to the fact that cilnidipine is less likely than other dihyropyridine CCBs to cause adverse effects such as tachycardia.[2,5,6]

Cilnidipine has been shown to inhibit the contractility of various isolated smooth muscles such as porcine coronary artery,[7] rat thoracic and tail arteries,[8] canine corpus cavernosum,[9] and rat lower oesophageal sphincter and aorta.[10] Calcium channels are present in the urinary bladder and are involved in physiological regulation of its contractility.[11,12] However, to date, the inhibitory effect of cilnidipine on the contractility of isolated detrusor muscle has not been studied. Hence, this study investigated the ability of cilnidipine to inhibit the potassium chloride (KCl)-induced contractility of the isolated caprine (goat) detrusor. Caprine detrusor was chosen because of easy availability and because its use has been standardized in our laboratory.[13–15] It was believed that the data from such a study could help facilitate the introduction of cilnidipine for the treatment of clinical conditions such as overactive bladder (OAB) requiring detrusor muscle relaxation. OAB is a chronic medical condition with a point prevalence of about 16.5% and has a major effect on the quality of life in affected men and women, influencing daily activities and social functions and interactions.[16] OAB has been defined as “urinary urgency, usually accompanied by frequency and nocturia, with or without urge incon-

* Address correspondence to: Jacob Peedicayil, MD, Department of Pharmacology & Clinical Pharmacology, Christian Medical College, Vellore 632002, India.
E-mail address: jpeedi@cmcvellore.ac.in (J. Peedicayil).

https://doi.org/10.1016/j.curtheres.2023.100717

tinence, in the absence of urinary tract infection or other obvious pathology."[16]

Methods

Tissue preparation

Fourteen caprine urinary bladder samples were obtained from a local butcher shop and transported to the pharmacology laboratory in physiological salt solution (PSS). The composition of the PSS was in millimoles: sodium chloride: 111.5, KCl: 4.6, magnesium sulfate: 1.16, sodium phosphate: 1.16, calcium chloride: 2.5, sodium bicarbonate: 21.9, and glucose: 11.1. In the laboratory, strips of detrusor measuring 10×3 mm were cut from the bladder samples as done previously in our laboratory.[13–15,17] The urothelium was removed by gentle scraping of the strips. The strips were mounted in an organ bath containing adequately oxygenated PSS maintained at a temperature of 37 °C. A resting tension of 10 mN (about 2.5 g) was applied to the suspended strip. The study was approved by our institutional review board and ethics committee (No. 11706; December 3, 2018).

Drugs

KCl was dissolved in double-distilled water to obtain a concentration of 149 mg/mL. KCl aliquot was prepared fresh each day. Cilnidipine (Sigma-Aldrich, St Louis, Missouri) was dissolved in dimethyl sulphoxide (DMSO) (Sigma-Aldrich) to give a 5 mg/mL stock solution. At concentrations routinely used to dissolve drugs, DMSO has been previously shown in our laboratory to not significantly influence the contractility of the isolated detrusor muscle.[12] FPL64716 (Sigma-Aldrich) was dissolved in DMSO to give a 10 μM stock solution. GV-58 (Santa Cruz Biotechnology, Dallas, Texas) was dissolved in double-distilled water to give a 4 μM stock solution.

Experimental procedure

Dose-response effects of cilnidipine

Following an equilibration period of 45 minutes, the tension was readjusted to 25 mN. The response of the detrusor strips to the administration of 80 mM KCl was then studied. An amount of 80 mM KCl is the standard concentration of KCl used in our laboratory for stimulating the contraction of the isolated detrusor.[13] After washing the bath, cilnidipine at a concentration of 20 μM was added to the organ bath and allowed to incubate for a period of 5 minutes. Then 80 mM KCl was added again and the contractile response was obtained. During each tracing, after drug administration, a contact time of 120 seconds was given, after which the tissue was washed till the baseline was attained. This procedure was then repeated with 2 higher concentrations of cilnidipine (40 and 60 μM).

Reversal of inhibitory effect of cilnidipine by calcium channel openers FPL64716 and GV-58

To confirm the mechanism of inhibitory effect of cilnidipine on the isolated detrusor, the following procedure was performed: the detrusor strip was first made to contract with 80 mM KCl with 40 μM cilnidipine. After washing out the KCl and cilnidipine and a rest period, the reversal agent FPL64716 (5μM), an L-type channel opener, was incubated with 40 μM cilnidipine in the organ bath for 10 minutes after which 80 mM KCl was added and the detrusor contraction was obtained. This process was repeated again using the N-type channel opener GV-58 (6.8 μM). The concentrations of FPL64716[18,19] and GV-58[20,21] used in the current study were based on previous studies.

Statistical Analysis

Calculation of height of contraction and area under the contractile curve

Contractility was computed by measuring the maximum height of contraction and the area under the contractile curve (AUCC) of the tracings, a method which we have standardized in our laboratory.[13–15,17] These parameters were calculated by scanning the tracings after each experiment and analysis using the software Image Tool (University of Texas Health Sciences Center at San Antanio, Texas).

Calculation of percent inhibition of KCl-induced contractility of detrusor by cilnidipine

The mean values of height of contraction and the AUCC after the administration of KCl were compared with the mean values of these parameter after the administration of KCl plus the 3 concentrations of cilnidipine (20, 40, and 60 μM) to determine the percent inhibition by the 3 concentrations of cilnidipine of KCl-induced detrusor contractility. The nonparametric test Wilcoxon signed-rank test was used for statistical analysis.

Calculation of percent inhibition of KCl-induced contractility by cilnidipine after administration of calcium channel openers

The mean percent inhibition of KCl-induced height of contraction and AUCC due to 40 μM cilnidipine plus the calcium channel agonists (FPL64716 or GV-58) was compared with the mean percent inhibition of these parameters due to KCl and 40 μM cilnidipine alone to determine whether the calcium channel openers reverse the effects of cilnidipine or not. The nonparametric test Wilcoxon signed-rank test was used for statistical analysis.

Results

The results of the effects of the 3 concentrations (20, 40, and 60 μM) of cilnidipine on KCl-induced contractility of isolated detrusor strips are shown in Table 1. All 3 concentrations of cilnidipine caused a dose-dependent and significant inhibitory effect of KCl-induced detrusor contractility. The results of the effects of the calcium channel openers on 80 mM KCl and 40 μM cilnidipine-induced detrusor contraction are shown in Table 2. Both calcium channel opener agents significantly reversed the inhibitory effect of cilnidipine on detrusor contractility because in their presence the mean percent inhibition of detrusor contractility decreased and the reduced mean percent inhibitions were statistically significant in comparison to the mean percent inhibition due to the administration of 40 μM cilnidipine and 80 mM KCl without the calcium channel opener. Representative tracings of the effect of cilnidipine on KCl-induced detrusor contractility and the effect of the calcium channel opener GV-58 on 40 μM cilnidipine's inhibition of KCl-induced detrusor contractility are shown in the Figure.

Discussion

As alluded to in the Methods section, in our laboratory we regularly use KCl to contract isolated smooth muscle such as the detrusor muscle. KCl is a very effective smooth muscle contractile agent that crosses the detrusor muscle cell membrane via positively charged potassium ion channels and causes depolarization of the cell membrane and activation of VGCC. This leads to a rise in cytosolic free calcium ions, calmodulin-dependent myosin light chain kinase activation, myosin light chain phosphorylation, and

Table 1
Inhibitory effects of cilnidipine on potassium chloride (KCl)-induced contractility of isolated caprine detrusor (n = 14 for each drug administration).*

Drug administration	% Inhibition of height		% Inhibition of AUCC	
	Mean (SEM)	*P value*†	Mean (SEM)	*P* value†
80 mM KCl + 20 μM cilnidipine	22.04 (3.31)	0.001	20.9 (2.36)	0.001
80 mM KCl + 40 μM cilnidipine	27.17 (2.45)	0.001	33.3 (1.13)	0.001
80 mM KCl + 60 μM cilnidipine	71.08 (4.38)	0.001	72.3 (4.94)	0.001

AUCC = area under the contractile curve.
* Values of percent inhibition were obtained by comparing values after administration of cilnidipine with values after prior administration of KCl only.
† The *P* values indicate that cilnidipine significantly lowered the height of contraction and the AUCC due to KCl.

Table 2
Reversal of inhibition of potassium chloride (KCl)-induced detrusor contractility by cilnidipine using calcium channel openers (n = 14 for each drug administration).*

Drug administration	% Inhibition of height		% Inhibition of AUCC	
	Mean (SEM)	*P* value	Mean (SEM)	*P* value
80 mM KCl + 40 μM cilnidipine + FPL64716	8.4 (1.91)	0.002†	6.94 (1.82)	0.005†
80 mM KCl + 40 μM cilnidipine + GV-58	8.9 (0.49)	0.001†	9.63 (0.63)	0.001†

AUCC = area under the contractile curve.
* Values of percent inhibition were obtained by comparing values after administration of KCl + 40 μM cilnidipine + calcium channel opener (FPL64716 in the upper panel; GV-58 in the lower panel) with values due to prior administration of KCl + 40 μM cilnidipine only.
† In comparison to mean percent inhibition due to 40 μM cilnidipine without calcium channel opener.

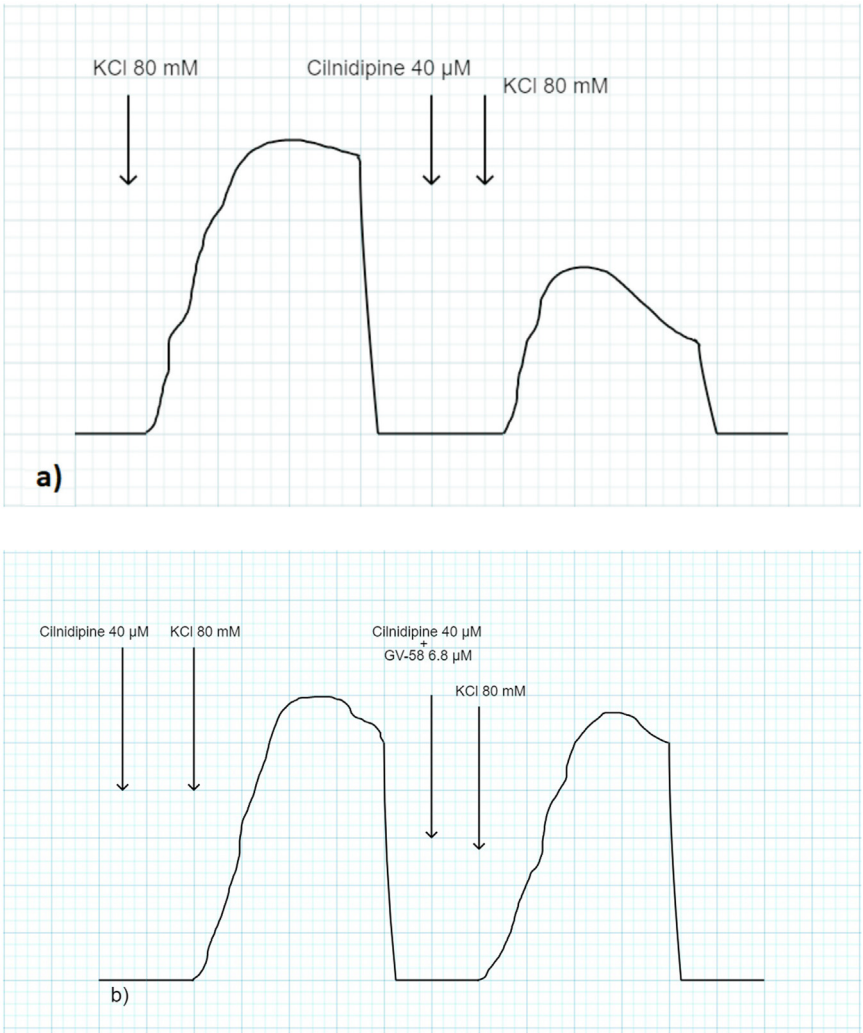

Figure. Representative traces from the study. (A) Contractile effect of 80 mM potassium chloride (KCl) before (left side) and after (right side) addition of 40 μM cilnidipine. (B) Contractile effect of 80 mM KCl + 40 μM cilnidipine before (left side) and after (right side) addition of GV-58.

muscle contraction.[22–25] Indeed, the fundamental function of detrusor muscle positively charged potassium ion channels is to precisely regulate and fine-tune calcium ion entry via VGCC.[25] Only the L-type VGCC is considered to be a major calcium ion influx pathway in smooth muscle.[26] KCl is not known to act via G-protein coupled receptors such as adrenergic (sympathetic) and muscarinic (parasympathetic) receptors, and indeed is used to bypass G-protein coupled receptors stimulation in experimental studies on smooth muscle.[24] However, KCl is also known to activate RhoA and Rho-kinase,[27] effects that would lead to smooth muscle contraction.[12]

This study has shown for the first time that cilnidipine inhibits the contractility of the isolated caprine detrusor muscle. We found that cilnidipine at all concentrations (20, 40, and 60 μM) used significantly inhibits the KCl-induced contractility of the detrusor in a dose-dependent manner (Table 1). Cilnidipine is known to be a smooth muscle relaxant, which is the basis for its use in hypertension. It is known to inhibit L- and N- type VGCC, and this is supported by our results because the L-type VGCC agonist FPL64716 and the N-type VGCC agonist GV-58 reversed cilnidipine's inhibitory effect on 80 mM KCl-induced detrusor contractility (Table 2). FPL64716 is believed to prolong both the opening of L-type VGCC during depolarization and the time course of inactivation upon repolarization. Patch clamp studies using rat ventricular myocytes have shown that FPL64716 increases the rate of calcium ion transients more than it increases the rate of rise of calcium ion transients. It also enhances the duration of tail currents upon repolarization.[18] GV-58 is a new molecule developed from the cyclin-dependent kinase inhibitor (R)-roscovitine.[20] It activates N-type, but not L-type, VGCC. GV-58 slows the deactivation of the N-type VGCC, thereby increasing total calcium ion flux, hence increasing neurotransmitter release from nerve terminals.[20] Both L- and N-type VGCCs are known to be present in the detrusor and take part in detrusor muscle contractility.[28,29] Hence, in the context of the current study, GV-58 causes increased detrusor muscle contractility by stimulating release of acetylcholine from parasympathetic nerve terminals, hence overcoming the relaxant effect of cilnidipine.[30] At the concentration of GV-58 used in the current study (6.8 μM), there is evidence from previous studies that it could have opened N-type VGCC.[21] To our knowledge, there is currently no evidence that GV-58 can induce smooth muscle contraction on its own. However, FPL64716 is known to contract isolated smooth muscle.[31] In the current study, neither FPL64716 nor GV-58, by themselves, contracted the isolated detrusor at the concentrations of these drugs that were used.

In previous studies on the effect of cilnidipine on isolated blood vessels, cilnidipine was found to exert its relaxant effect at nanomolar concentrations.[7,8] However, in the current study it was found that cilnidipine relaxes the detrusor muscle at micromolar concentrations (Table 1). A possible explanation for this finding could be the differences in concentrations of subtypes of VGCC (L, N, and T) between vascular muscle and the detrusor muscle. It is known that the tissue distribution of VGCC varies between tissues.[32,33] Moreover, there can be differences in the potency of CCBs for VGCC in different tissues.[34]

Currently used drugs for treating OAB include antimuscarinic drugs and β_3 receptor agonists.[12] However, the currently used drugs have adverse effects such as antimuscarinic effects and some patients do not respond to currently used drugs. There is also a high rate of patient dissatisfaction and stopping of present drug treatments.[35] Hence, newer alternatives will be useful for the treatment of OAB. One such class of new drugs that could be useful for treating OAB is CCBs.[12] Our results suggest that cilnidipine is such a CCB. Cilnidipine can be administered orally to patients and has a good pharmacokinetic profile.[1,3] The safety profile of cilnidipine is also good with no major adverse effects reported to date.[1,3] Hence, in the light of the above data, cilnidipine is a potential new candidate for the treatment of conditions such as OAB that require relaxation of the detrusor muscle. The tissue concentrations at which cilnidipine relaxes the isolated detrusor in this study (20, 40, and 60 μM) are clinically achievable after systemic administration to patients such as oral and parenteral administration.[17] Because cilnidipine has the potential to cause cardiovascular adverse effects, it could be used at relatively low doses with other suitable drugs for OAB such as anticholinergics. In this context, we have shown in our laboratory that when combined with a low concentration of the anticholinergic oxybutynin (5 μM), a relatively low concentration of cilnidipine (20 μM), significantly inhibits the contractility of the isolated caprine detrusor.[36]

A limitation of this study is that a more rigorous quantification of the effects of the agonists and antagonists on the VGCC of the detrusor muscle could have been done. For instance, the Schild plot could have been made to calculate pA_2 values for cilnidipine.[37] pA_2 refers to the affinity of an antagonist for its receptor. In this regard, Yamanishi et al[38] determined the pA_2 values for β-receptor antagonists in the isolated porcine detrusor muscle. However, as mentioned in the Introduction, we have standardized the way of quantifying effects of agonists and antagonists by determining the percent inhibition of agonist-induced contractility by antagonists and our work has been published in peer-reviewed and indexed journals.[13–15,17]

Conclusions

This study has shown for the first time that the fourth-generation CCB cilnidipine, at suitably low concentrations, inhibits the contractility of the isolated detrusor. Cilnidipine could be evaluated for the treatment of clinical conditions requiring relaxation of the detrusor such as OAB.

Declaration of Competing Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Acknowledgments

This study was funded by an intramural research grant. Each author was involved in the designing of the study. S. A. Maria did the laboratory work. A. Kumar prepared the Figure. J. Peedicayil wrote the manuscript, which was read and approved by each author.

References

1. Xu G, Wu H, Du B, Qin L. The efficacy and safety of cilnidipine on mild to moderate essential hypertension: A systematic review and meta-analysis of randomized controlled trials in Chinese patients. *Cardiovasc Hematol Disord Drug Targets*. 2012;12:56–62.
2. Chandra KS, Ramesh G. The fourth- generation calcium channel blocker: Cilnidipine. *Indian Heart J*. 2013;65:691–695.
3. Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. *Pharmacol Rev*. 2015;57:821–870.
4. Lanzetti S, Di Biase V. Small molecules as modulators of voltage-gated calcium channels in neurological disorders: State of the art and perspectives. *Molecules*. 2022;27:1312.
5. Uneyama H, Uchida H, Konda T, Yoshimoto R. Cilnidipine: Preclinical profile and clinical evaluation. *Cardiovasc Drug Rev*. 1999;17:341–357.
6. Takahara A. Cilnidipine: A new generation Ca channel blocker with inhibitory action on sympathetic neurotransmitter release. *Cardiovasc Ther*. 2009;27:124–139.
7. Leung HS, Yao X, Leung FP, et al. Cilnidipine, a slow- acting calcium channel blocker, induces relaxation in porcine coronary artery: Role of endothelial nitric oxide and $[Ca^{2+}]i$. *Br J Pharmacol*. 2006;147:55–63.
8. Nap A, Mathy MJ, Balt JC, Pfaffendorf M, Van Zweiten PA. The evaluation of the N-type channel blocking properties of cilnidipine and other voltage-dependent calcium antagonists. *Fundam Clin Pharmacol*. 2004;18:309–319.

9. Okamura T, Fujioka H, Ayajiki K. Effects of calcium antagonists on the nitrergic nerve function in canine corpus cavernosum. *Jpn J Pharmacol*. 2001;87:208–213.
10. Takahara A, Nozaki S, Ishiguro A, et al. Selectivity of Ca^{2+} channel blockers for dilator actions on the isolated lower esophageal sphincter and aorta from rats. *J Pharmacol Sci*. 2018;137:98–100.
11. Luptak J, Kocmalova M, Franova S, et al. Involvement of calcium regulating ion channels in contractility of human isolated urinary bladder. *Gen Physiol Biophys*. 2018;37:391–398.
12. Joseph S, Maria SA, Peedicayil J. Drugs currently undergoing preclinical or clinical trials for the treatment of overactive bladder: A review. *Curr Ther Res Clin Exp*. 2022;96:100669.
13. Kumar A, Prabha R, Paul T, et al. Tramadol inhibits the contractility of isolated caprine detrusor muscle. *Auton Autacoid Pharmacol*. 2012;32:15–22.
14. Dhruva A, Hamsavardhini V, Kamatham S, et al. Avanafil inhibits the contractility of the isolated caprine detrusor muscle. *Int J Appl Basic Med Res*. 2019;9:231–235.
15. George N, Shiny PJ, Miriam J, Nancy CA, Dhanasekar KR, Peedicayil J. Inhibitory effect of anticholinergics on the contraction of isolated caprine urinary bladder detrusor muscle. *Auton Autacoid Pharmacol*. 2010;30:173–177.
16. Leron E, Weintraub AY, Mastrolia SA, Schwarzman P. Overactive bladder syndrome: Evaluation and management. *Curr Urol*. 2018;11:117–125.
17. Faruqui AR, Mathai J, George J, Peedicayil J, Ernest K, Neelakantan N. Inhibitory effect of nicorandil on the contraction of ilsolated human urinary bladder detrusor muscle. *Methods Find Exp Clin Pharmacol*. 2008;30:363–366.
18. Ghais NS, Zhang Y, Grace AA, Huang CL-H. Arrhythmogenic actions of the Ca^{2+}channel agonist FPL-64716 in Langendorff-perfused murine hearts. *Exp Physiol*. 2008;94:240–254.
19. Zhao J, Wang Y, Gao J, Jing Y, Xin W. Berberine mediated positive inotropic effects on rat hearts via a Ca^{2}-dependent mechanism. *Front Pharmacol*. 2020;11:821.
20. Tarr TB, Lacomis D, Reddel SW, et al. Complete reversal of Lambert-Eaton myasthenic syndrome synaptic impairment by the combined use of a K^{+} channel blocker and a Ca^{2+} channel agonist. *J Physiol*. 2014;592:3687–3696.
21. Wu M, White HV, Boehm BA, et al. New Cav2 calcium channel gating modifiers with agonist activity and therapeutic potential to treat neuromuscular disease. *Neuropharmacology*. 2018;131:176–189.
22. Eschenhagen T. Treatment of ischaemic heart disease. In: Brunton LL, Knollman BC, eds. *The Pharmacological Basis of Therapeutics*. New York: McGraw Hill; 2023:607–624.
23. Zamponi GW, Striessnig J, Koschak A, Dolphin AC. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. *Pharmacol Rev*. 2015;67:821–870.
24. Ratz PH, Berg KM, Urban NH, Miner AS. Regulation of smooth muscle calcium sensitivity: KCl as a calcium-sensitizing stimulus. *Am J Physiol Cell Physiol*. 2005;288:C769–C783.
25. Petkov GV. Role of potassium ion channels in detrusor smooth muscle function and dysfunction. *Nat Rev Urol*. 2012;9:30–40.
26. Karaki H, Ozaki H, Hori M, et al. Calcium movements, distribution, and functions in smooth muscle. *Pharmacol Rev*. 1997;49:157–230.
27. Janssen LJ, Tazzeo T, Zuo J, Pertens E, Keshavjee S. KCl evokes contraction of airway smooth muscle via activation of RhoA and Rho kinase. *Am J Physiol Lung Cell Mol Physiol*. 2004;287:L852–L858.
28. Engin S, Barut EN, Erac Y, Sari S, Kadioglu M. The inhibitory effect of escitalopram on mouse detrusor contractility: The role of L-type calcium channels. *Toxicol Appl Pharmacol*. 2023;461:116408.
29. Igawa Y, Kumano S, Aizawa N, et al. Changes in the function and expression of T-type and N-type calcium channels in the rat bladder after bladder outlet obstruction. *J Urol*. 2014;191:1159–1167.
30. Waterman SA. Multiple types of voltage-gated calcium channel mediate transmitter release from parasympathetic neurons in the mouse bladder. *J Neurosci*. 1996;16:4155–4161.
31. Rampe D, Dage RC. Functional interactions between two Ca2+ channel activators, (S)-Bay K 8644 and FPL64176, in smooth muscle. *Mol Pharmacol*. 1992;41:599–602.
32. Biel M, Hullin R, Freundner S, et al. Tissue-specific expression of high-voltage-activated dihydropyridine-sensitive L-type calcium channels. *Eur J Biochem*. 1991;200:81–88.
33. Graf EM, Bock M, Heubach JF, et al. Tissue distribution of a human Ca v 1.2 alpha1 subunit splice variant with a 75 bp insertion. *Cell Calcium*. 2005;38:11–21.
34. Godfraind T. Discovery and development of calcium channel blockers. *Front Pharmacol*. 2017;8:286.
35. Fontaine C, Papworth E, Pascoe J, Hashim H. Update on the management of overactive bladder. *Ther Adv Urol*. 2021;13:1756282211039034.
36. Joseph S. *Study of the Effects of Oxybutynin, Mirabegron and Cilnidipine in Different Dual Combinations on Isolated Detrusor Muscle* [submitted thesis]. Chennai, Tamil Nadu, India: Tamil Nadu Dr MGR Medical University; 2023.
37. Schild HO. pA, a new scale for the measurement of drug antagonism. *Br J Pharmacol Chemother*. 1947;2:189–206.
38. Yamanishi T, Chapple CR, Yasuda K, Yoshida K, Chess-Williams R. The role of β_3 - adrenoceptors in mediating relaxation of porcine detrusor muscle. *Br J Pharmacol*. 2002;135:129–134.