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Brief Communication

Stimulation of imidazoline type 2 receptors induces contractions of the mouse urinary bladder through KATP channel modulation

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Abstract: Imidazoline type 2 receptor (I2R) is expressed in the urinary bladder and is implicated in smooth muscle regulation. However, its role under resting conditions remains unclear. This study investigated the effects of I2R stimulation on bladder contractility under non-precontracted conditions and explored the involvement of ATP-sensitive potassium (KATP) channels. Female mouse bladder strips were mounted in an organ bath and exposed to the selective I2R agonist 2-(2-benzofuranyl)-2-imidazoline (2BFI; 1-100 μM). To evaluate I2R and KATP involvement, strips were pretreated with the I2R antagonist BU224 or the KATP channel opener pinacidil. Contractile activity was quantified as the area under the curve (AUC). 2BFI induced concentration-dependent increases in contractile activity. BU224 and pinacidil attenuated 2BFI-induced contractions, with significant inhibition at higher concentrations. I2R stimulation induced bladder contraction under resting conditions. The attenuation by pinacidil suggests functional involvement of KATP channel modulation. These findings suggest a role of I2R in bladder function and potential relevance to underactive bladder.

Keywords: Imidazoline type 2 receptor, urinary bladder, ATP-sensitive potassium channel, organ bath, detrusor underactivity

Introduction

Imidazoline receptors are classified into three subtypes (I1, I2, and I3), which are distinct from adrenergic receptors and are widely distributed in various tissues, including the central nervous system and peripheral organs [1]. Among these subtypes, the imidazoline type 2 receptor (I2R) has been implicated in the regulation of smooth muscle function and has been identified in the urinary bladder [1, 2]. Previous studies, including ours, have demonstrated that activation of I2R can induce relaxation of bladder smooth muscle in precontracted conditions [2, 3], suggesting a role in the modulation of bladder function. I2R has also been implicated in the regulation of nociception and central nervous system function [4, 5]. However, the effects of I2R stimulation on bladder contractility under resting, non-precontracted conditions remain unclear. Since physiological bladder function involves both storage and voiding phases, it is important to understand how I2R contributes to bladder activity in different functional states.

Potassium channels, particularly ATP-sensitive potassium (KATP) channels, play a crucial role in regulating detrusor smooth muscle excitability [6]. Modulation of KATP channels influences membrane potential and thereby alters bladder contractility. Opening of KATP channels increases potassium efflux, hyperpolarizes the membrane, and reduces detrusor excitability, whereas inhibition promotes membrane depolarization and contraction [6-8]. Previous stud-

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ies suggest that I2R-mediated relaxation in precontracted bladder tissue may involve cyclic adenosine monophosphate/protein kinase A-dependent KATP channel activation [2], whereas imidazoline compounds can inhibit KATP currents and depolarize other smooth muscles [9]. However, the relationship between I2R signaling and KATP channel activity in bladder smooth muscle has not been fully elucidated. Underactive bladder (UAB), which is often associated with detrusor underactivity, is characterized by reduced bladder contractility and impaired bladder emptying [10]. Recent studies have further emphasized the clinical relevance of UAB and detrusor underactivity, particularly in aging populations [11, 12]. Despite its clinical importance, effective pharmacological treatments for UAB remain limited. Therefore, identifying mechanisms that enhance bladder contractility may have therapeutic implications for patients with UAB.

The present study aimed to investigate the effects of I2R stimulation on bladder contractility under non-precontracted conditions and to determine whether modulation of KATP channels is involved in this response.

Materials and methods

Female C57BL/6N mice aged 9-10 weeks and weighing 18-22 g were obtained from Envigo (Frederick, MD, USA). Mice were housed three to four per cage at 22-24°C under a 12-hour light/dark cycle (lights on at 7:00 AM), with food and water available ad libitum. Animals were euthanized by carbon dioxide inhalation using 100% CO_2 supplied from a gas cylinder at a controlled fill rate of 30-70% of the chamber volume per minute, followed by cervical dislocation. Urinary bladders were harvested and cut into two longitudinal strips. Each bladder strip was used only once. When two strips were obtained from the same mouse, they were assigned to different treatment groups; therefore, no treatment group included more than one strip from the same animal. Sample sizes differed among groups because some strips were not included in the analysis due to unstable baseline recordings. Bladder muscle strips were mounted longitudinally in a vertical double-jacketed organ bath (Model 159920, Radnoti, LLC, Monrovia, CA, USA) connected to an isometric force transducer (Model 159901A, Radnoti, LLC); signals were recorded using a PowerLab data-acquisition system with LabChart software (ADInstruments, Colorado Springs, CO, USA). Each bath contained 15 mL of Krebs solution (in mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2, and dextrose 11.7). Krebs solution reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). The solution was maintained at 37°C and continuously bubbled with 95% O_2 and 5% CO_2. A resting tension of 10 mN (1 g) was applied, and tissues were allowed to equilibrate for 1-2 hours before experiments.

2-(2-Benzofuranyl)-2-imidazoline (2BFI) hydrochloride (catalog no. 0348; Tocris Bioscience, Bristol, UK) is a selective I2R agonist, whereas BU224 hydrochloride (catalog no. 0725; Tocris Bioscience, Bristol, UK) is an I2R antagonist and pinacidil monohydrate (catalog no. 15416; Cayman Chemical, Ann Arbor, MI, USA) is a KATP channel opener.

To evaluate the effects of I2R stimulation under non-precontracted conditions, 2BFI (1, 10, and 100 μM) was applied non-cumulatively, with a separate bladder strip used for each concentration. To assess the involvement of I2R and KATP channels, bladder strips were pretreated with BU224 (10 or 100 μM) or pinacidil (10 or 100 μM) for 15 minutes before the application of 2BFI (100 μM). Control strips received 2BFI (100 μM) without BU224 or pinacidil pretreatment. 2BFI and BU224 were added directly to the organ bath to achieve the indicated final concentrations. Pinacidil monohydrate was dissolved in dimethyl sulfoxide (DMSO) before being added to the organ bath. The DMSO-only vehicle control group was not included. Contractile activity was continuously recorded, and changes in contractile responses after drug administration were evaluated by comparing 2BFI-induced contractions with baseline activity.

Contractile activity was quantified as the area under the curve (AUC) during a 5-minute baseline period immediately before treatment and during the 10-15-minute period after 2BFI application. The percent change was calculated as [(post-2BFI AUC - baseline AUC)/baseline AUC] × 100.

All animal procedures were conducted in accordance with the ARRIVE guidelines and the NIH Guide for the Care and Use of Laboratory

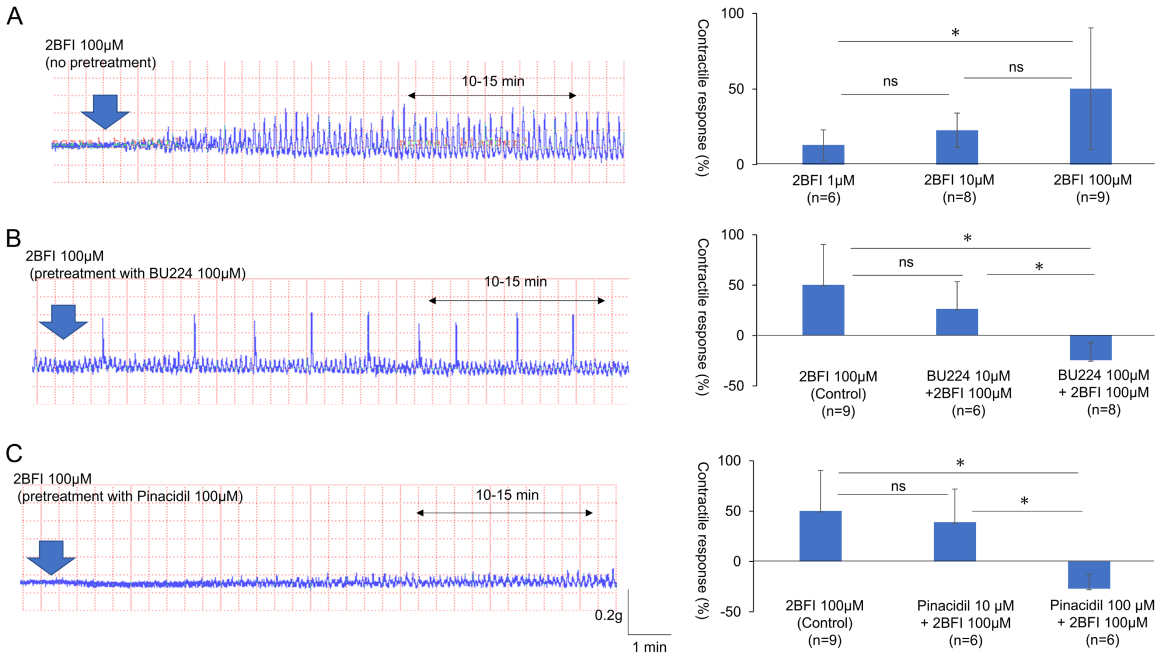

Figure 1. Effects of imidazoline type 2 receptor (I2R) stimulation on contractile responses of mouse bladder muscle strips. (A) Non-cumulative effects of 2-(2-benzofuranyl)-2-imidazoline (2BFI; 1, 10, and 100 μM) under non-precontracted conditions. Sample sizes: n = 6, 8, and 9 strips, respectively. Contractile activity increased with increasing concentrations of 2BFI. Although the response at 100 μM tended to be higher than that at 10 μM, the difference was not statistically significant (P = 0.07). (B) Effects of the I2R antagonist 2-(4,5-dihydroimidazol-2-yl)quinoline (BU224). Pretreatment with BU224 (10 or 100 μM) for 15 minutes attenuated contractions induced by 2BFI (100 μM). Sample sizes: n = 9 for the 2BFI control, n = 6 for BU224 10 μM, and n = 8 for BU224 100 μM. A significant inhibitory effect was observed at 100 μM, whereas 10 μM showed no significant effect. (C) Effects of the adenosine triphosphate-sensitive potassium (KATP) channel opener pinacidil. Pretreatment with pinacidil (10 or 100 μM) for 15 minutes reduced contractile responses induced by 2BFI (100 μM). Sample sizes: n = 9 for the 2BFI control and n = 6 for each pinacidil group. The same 2BFI control dataset is shown in (B and C). Data are mean ± standard deviation. *P < 0.05; ns, not significant; two-sided Mann-Whitney U test.

Animals, and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pittsburgh (Approval No. 22061313).

Data are presented as mean ± standard deviation to describe the magnitude and variability of the responses and to maintain consistency with the graphical presentation. Statistical comparisons were performed using two-sided Mann-Whitney U tests. The experimental unit was the bladder strip, and n indicates the number of strips analyzed. Because the group sizes were small (n = 6-9), data distribution normality would not be reliably assessed; therefore, non-parametric analyses were used. In addition, since this study is based on the exploratory approach, *p*-values here should be considered descriptive, but not as hypothesis testing. Statistical analyses were performed using EZR version 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [13]. A value of $P < 0.05$ was considered statistically significant.

Results

Application of 2BFI induced concentration-dependent contractile responses in bladder muscle strips under non-precontracted conditions (**Figure 1A**). Contractile activity increased with increasing concentrations of 2BFI, with 10 and 100 μM showing greater responses than 1 μM. Although the response at 100 μM appeared higher than that at 10 μM, the difference did not reach statistical significance (P = 0.07), indicating a trend toward a dose-dependent increase.

Pretreatment with the I2R antagonist BU224 significantly reduced the contractile responses induced by 2BFI (100 μM) (**Figure 1B**). While

pretreatment with BU224 at 10 μM showed a reduction in 2BFI-induced contractile activity, the effect was not statistically significant. In contrast, 100 μM BU224 markedly attenuated the contractile responses induced by 2BFI, demonstrating a concentration-dependent inhibitory effect and supporting the involvement of I2R in modulation of bladder contractility.

Similarly, pretreatment with the KATP channel opener pinacidil significantly attenuated the contractile responses induced by 2BFI (100 μM) (**Figure 1C**). Although 10 μM pinacidil showed a tendency to reduce 2BFI-induced contractile activity, this effect did not reach statistical significance. In contrast, 100 μM pinacidil significantly suppressed the contractile responses induced by 2BFI, indicating a dose-dependent inhibitory effect of KATP channel activation.

Taken together, these findings demonstrate that I2R stimulation induces bladder contraction under non-precontracted conditions and that I2R-mediated contractile responses of bladder strips are modulated, at least in part, through KATP channel activity.

Discussion

In this study, we demonstrated that stimulation of I2R induces bladder contraction in mouse bladder muscle strips under non-precontracted conditions. Furthermore, this contractile response was attenuated by both an I2R antagonist and a KATP channel opener, suggesting that I2R-mediated contraction is at least partially dependent on KATP channel modulation.

Previous studies have reported that I2R activation induces relaxation in precontracted bladder tissues [2]. In contrast, our findings demonstrated that I2R stimulation induces contraction under resting bladder conditions. This discrepancy suggests that the functional role of I2R in bladder smooth muscle may depend on the contractile state of the bladder.

The involvement of KATP channels in smooth muscle regulation has been well established [6-8]. Activation of KATP channels typically leads to membrane hyperpolarization and muscle relaxation, whereas inhibition promotes membrane depolarization and contraction. In addition, imidazoline receptor stimulation has been reported to induce smooth muscle contraction through membrane depolarization mediated by inhibition of KATP channels [9]. Our results, showing that pinacidil attenuates 2BFI-induced contraction of bladder strips, are consistent with the possibility that I2R stimulation reduces KATP channel activity and thereby enhances bladder smooth muscle contractility under resting conditions. However, KATP currents and membrane potentials were not directly measured; therefore, the present study demonstrates functional modulation by a KATP channel opener, but does not prove direct inhibition of KATP channels by I2R stimulation.

These findings may have implications for UAB with detrusor underactivity, a condition characterized by reduced detrusor contractility and impaired bladder emptying [10]. Chronic bladder ischemia, which is often associated with aging-related vascular changes, has been recognized as a contributor to detrusor underactivity [14], and I2R activation has been suggested to exert neuroprotective effects under ischemic conditions [15]. Detrusor underactivity is a multifactorial condition involving both myogenic and neurogenic mechanisms [16]. Recent studies, including our own previous work, have demonstrated that I2R activation improves voiding efficiency in a spinal cord injury mouse model [3]. Although spinal cord injury is not a pure model of underactive bladder, it shares characteristics of impaired bladder emptying [17]. Together with our present findings showing enhanced bladder contractility in an organ bath system, I2R activation may have therapeutic potential for improving voiding function in conditions associated with detrusor underactivity. Because I2R activation enhances bladder contraction under resting conditions, modulation of this pathway may represent a promising therapeutic strategy for improving bladder emptying in patients with detrusor underactivity.

This study has several limitations. First, the experiments were conducted using an in vitro organ bath system; therefore, in vivo validation is needed to confirm the physiological relevance of these findings. Second, the intracellular signaling pathways linking I2R activation to KATP channel modulation remain to be fully elucidated. Future studies using animal models of detrusor underactivity will be necessary

to clarify the translational significance of these findings. Third, only female mice were used, and sex-related differences in lower urinary tract function should be considered when interpreting these findings [18].

Conclusion

Stimulation of imidazoline type 2 receptors induces bladder contraction in mouse bladder muscle strips under non-precontracted conditions. The attenuation of this response by pinacidil suggests that KATP channel modulation is involved. These findings suggest a state-dependent role of I2R in bladder function and indicate that targeting I2R may represent a potential therapeutic strategy for UAB with detrusor underactivity.

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Disclosure of conflict of interest

None.

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