H2O2 enhances spontaneous contractions of human-bladder strips via activation of TRPA1 channels on sensory nerves and release of substance P and PGE2

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Abstract

Abstract Background and purpose: Several studies have indicated that reactive oxygen species (ROS) can lead to detrusor overactivity (DO), but the underlying mechanisms are not known. Hydrogen dioxide (H2O2) is used commonly to investigate the effects of ROS. In present study, we aimed to investigate the effects of H2O2 on phasic spontaneous bladder contractions (SBCs) of isolated human-bladder strips (iHBSs) and the underlying mechanisms. Experimental approach: Samples of bladder tissue were obtained from 26 patients undergoing cystectomy owing to bladder cancer. SBCs of iHBSs were recorded in organ-bath experiments. Immunofluorescence staining was conducted to confirm the TRPA1 expression on sensory nerves. Key results: H2O2 (1 μM–10mM) concentration-dependently increased the SBCs of iHBSs. These enhancing effects could be mimicked by an agonist of transient receptor potential (TRP)A1 channels (allyl isothiocyanate) and blocked with an antagonist of TRPA1 channels (HC030031; 10μM). H2O2 induced enhancing effects also could be attenuated by desensitizing sensory afferents with capsaicin (10μM), blocking nerve firing with TTX (1μM), blocking neurokinin effects with NK2 receptor antagonist (SR48968, 10μM), and blocking PGE2 synthesis with indomethacin (10μM), respectively. Conclusions and implications: Our study: (i) suggests activation of TRPA1 channels on bladder sensory afferents, and then release of substance P or PGE2 from sensory nerve terminals, contribute to the H2O2-induced enhancing effects on SCs of iHBSs; (ii) provides insights for the mechanisms underlying ROS leading to DO; (iii) indicates that targeting TRPA1 channels might be the promising strategy against overactive bladder in conditions associated with excessive production of ROS.

1. Introduction

Overactive bladder (OAB) is a common condition affecting millions of people worldwide. It has been defined by the International Continence Society as a syndrome characterized by urgency (with or without urge incontinence), usually with frequency, or nocturia [1, 2]. It has been proposed that OAB symptoms are closely associated with exaggerated spontaneous bladder contractions (SBCs), referred to as “detrusor overactivity” (DO)[3, 4] or “no-voiding contraction.” Consequently, DO is the target for medical treatment of the storage symptoms of OAB[5]. Thus, unraveling the underlying mechanisms of DO is valuable to identify novel drug targets for OAB treatments[3, 5].

OAB is prevalent in patients or animal models with bladder outlet obstruction (BOO)[6], injury to the spinal cord [7], diabetes mellitus[8], chronic ischemia[9], or aging[10]. These pathologic conditions are characterized by enhanced oxidative stress and excessive accumulation of reactive oxygen species (ROS) due to ischemia, ischemia–reperfusion, or inflammation[5, 11-13]. Thus, oxidative stress and the resulting increased levels of ROS has been proposed to be an important mechanism for DO development[14-16].
Hydrogen dioxide (H$_2$O$_2$) is the most likely ROS involved in signal transduction, and has been used commonly to investigate the effects of ROS[15, 17]. In support of the role of ROS in DO, an in vivo study in rats showed that intravesical instillation of H$_2$O$_2$ (0.003–0.3%) could induce OAB[15]. Intravesical injection of H$_2$O$_2$ has been utilized to establish an animal model of OAB[18]. In vitro studies in rats showed that H$_2$O$_2$ increased the spontaneous contractions (SBCs) of isolated bladder strips in a concentration-dependent manner[17, 19]. However, the exact mechanisms underlying how excessive ROS leads to DO are not known. Furthermore, most of these types of studies have been from animal bladders, and studies on the human bladder are lacking.

Pathologic DO has been considered to be an amalgamation of small and localized asynchronous SBCs, which could be measured in vivo during normal filling of the bladder and could also be recorded in vitro in isolated whole bladder or bladder strips from animal and human sources[20]. Studies on the origin or modulation of SBCs may help to unravel the underlying mechanisms of pathologic DO[20]. β3-adrenoceptor agonists which have been used to treat OAB were shown to suppress SBCs in human-bladder strips[21]. In vitro recording of SBCs has indicated that SBCs could be increased by nerve stimulation, muscarinic agonists[22], nicotinic ligands, adenosine triphosphate (ATP), and substance P (SP)[23], and decreased by noradrenaline and calcitonin gene-related peptide (CGRP)[24, 25]. The modulatory effects of SP and CGRP (the two neuropeptide transmitters released from bladder sensory afferents) implicate a role of the axon reflex or the “efferent” role of sensory afferents in SBCs regulation[25, 26].

Transient receptor potential (TRP)A1 channels are expressed on C-fiber bladder afferents [27, 28]. TRPA1 activation by intravesical instillation of TRPA1 activators such as allyl isothiocyanate (AITC) can initiate DO[27]. In vitro recordings have indicated that AITC enhanced the SBCs of isolated bladder strips from rats or guinea pigs by activating TRPA1 on bladder sensory afferents[29-31]. Those studies suggest a close correlation between activation of TRPA1 on sensory afferents and DO. Furthermore, Andrade et al[31] found that the release of SP and prostaglandin (PG)E2 contributed to AITC-mediated enhancement of SBCs, which suggests that TRPA1 activation-induced DO may result from the efferent role of bladder sensory afferents.

TRPA1 channels expressed on sensory afferents have been shown to be the main molecular target for H$_2$O$_2$[28, 32]. In rats or mice, H$_2$O$_2$ administration has been demonstrated to evoke increases in inward current or the intracellular calcium (Ca$^{2+}$) concentration in a subset of dorsal-root ganglia neurons that were also responsive to TRPA1 agonists[32-34]. H$_2$O$_2$ application evoked a long-lasting firing of most capsaicin-sensitive high-threshold afferents via TRPA1 activation in the guinea-pig bladder[28].

Given the excitatory role of H$_2$O$_2$ on bladder sensory afferents and the modulatory role of bladder sensory afferents on SBCs, we hypothesized that TRPA1 activation by ROS induced the release of neurotransmitters from peripheral sensory nerve terminals of the bladder contributed to DO. There are pronounced differences in SBCs patterns between humans and animals[35]. Also, studies examining the effects of ROS on the contractile activity of the bladder have been conducted almost exclusively on laboratory animals. Hence, in the present study we investigated H$_2$O$_2$-induced effects on bladder contractile activity and the mediating mechanisms using the human bladder tissue.

2. Materials and Methods

2.1 Ethical approval of the study protocol

The experimental protocol was approved (KYLL-2023LW038) by the Ethics Committee of the Second Hospital, Cheeloo College of Medicine in Shandong University (Jinan, China). Our study was conducted in accordance with the Declaration of Helsinki 1964 and its later amendments. All patients provided written informed consent.

2.1 Samples of human bladder

Samples of human bladder were obtained from 26 patients (11 women, 15 men; patients mean age = 49 ± 10 years; age range = 39–59 years) undergoing cystectomy owing to bladder cancer in the Second Hospital or Qilu Hospital of Shandong University. Tissues were examined by a uropathologist. No histological
signs of inflammation or carcinoma were observed. After removal, each specimen was transported immediately to the laboratory for organ-bath experiments. A portion of each tissue was placed in formalin for immunofluorescence experiments.

2.3 Organ-bath experiments

All samples were taken from the bladder body. From each patient, 5–10 longitudinal bladder strips (length = 20 ± 1.5 mm; width = 8–9 mm) were studied. Bladder strips were placed in warm Krebs solution (composition in mM: NaCl, 118; KCl, 4.7; CaCl₂, 1.9; MgSO₄, 1.2; NaHCO₃, 24.9; KH₂PO₄, 1.2; glucose, 11.7; pH = 7.4) and bubbled with 95% O₂ and 5% CO₂. Strips were tied at each end using a fine thread, mounted in a vertical organ bath in oxygenated Krebs solution (volume = 20 mL), and heated to 37 °C in a circulating warm water-bath. The longitudinal tension of the isolated preparations was recorded continuously with an isometric transducer and then processed with Lab Chart 7 (AD Instruments, New South Wales, Australia). Tissues were stretched to a baseline tension of 15 mN (1.5 g). Contractions were allowed to equilibrate for 30–40 min, until the appearance of stable SBCs. In strips with no SBCs, carbachol (1–10 nM) was applied to initiate SBCs. Strips that had a spontaneous or carbachol-evoked SBCs were considered as having “good” viability. All the strips from the 26 patients had good viability.

In a pilot study, we found that repeated application of high concentrations ([?]100 μM) of H₂O₂ in the same strip induced desensitization (supplementary figure 1). Thus, the noncumulative concentration–response relationship of H₂O₂ was determined. On completion of experiments, threads were removed and strips were weighed after water absorption with tissue paper.

2.4 Immunofluorescence staining

Immunofluorescence staining was conducted to confirm the TRPA1 expression on sensory nerves. Bladder tissues were fixed with 4% formalin for >24 h. Bladder tissues were embedded in paraffin and then cut in the transverse direction into sections of thickness 8 μm. Sections were deparaffinized, rehydrated, and followed by antigen retrieval for 10 min. Then, sections were blocked with 5% albumin from goat serum in a 37 °C oven for 1 h, followed by incubation with mixed primary antibodies (TRPA1, 1:100, Alomone labs; Substance P, SANTA CRUZ, 1:100) overnight at 4 °C. Primary antibodies were visualized with Alexa Fluor 594-conjugated goat anti-mouse IgG (H+L; diluted 1:200 in phosphate-buffered saline, Elabscience Biotechnology Co., Ltd., Wuhan, China) or fluorescein-conjugated goat anti-rabbit IgG (H+L; diluted 1:50). Sections were analyzed by Confocal laser scanning microscope ZEISS Observer.Z1 (Carl Zeiss Microscopy GmbH, Baden Wurttemberg, Germany). Images were acquired with the ZEN 2.1 (blue version) (Carl Zeiss Microscopy GmbH, Baden-Wurttemberg, Germany). Control staining was performed with PBS rather than primary antibodies.

2.5 Drugs

H₂O₂, HC-03003, AITC, capsaicin, tetrodotoxin (TTX) and Carbachol were purchased from Sigma-Aldrich (Darmstadt, USA). While PGE₂, SP and a selective antagonist of neurokin-2 receptors (NK2Rs), SR 48968, were sourced from MCE (New Jersey, USA).

Stock solution of capsaicin were prepared in 100% ethanol and stored at -20 °C until use. AITC, SR 48968, PGE₂, and SP solutions were made in dimethyl sulfoxide and diluted immediately before use. TTX was dissolved in deionized water. The final concentrations of ethanol or dimethyl sulfoxide were <0.1%. In all experimental groups, at least one parallel control experiment was carried out in vehicle. The vehicles we used had no pharmacological effects on the tone of preparations or agonist-induced contractions. Chemicals were used at concentrations shown to be effective in preliminary experiments, or at concentrations used in studies published previously[29, 30].

2.6 Data analyses

Data are the mean ± SEM. SBCs was measured at 15-min intervals immediately before (control) and after drug application. The response to H₂O₂ was evaluated as the percent increase in the area under the curve.
(AUC, an integrative of the amplitude, baseline tension, and frequency of phasic contractions) as well as the net increase in baseline tension (Δg) from the control. Δg was normalized with the wet weight of strips (Δg/g). Concentration–response curves were fitted with the Hill equation: Percent enhancement = \( \frac{\text{MAX}_{\text{enhancement}}}{\text{drug concentration} + \text{EC}_{50}} \), where MAX_{\text{enhancement}} is maximal % enhancement, drug concentration is the agonist concentration, EC_{50} is the half-maximal effective concentration, and n is the Hill coefficient. Statistical significance was tested with the one-sample t-test or paired t-test (two-tailed). Statistical analyses were undertaken with Excel (Microsoft, Redmond, WA, USA) and Prism 8 (GraphPad, La Jolla, CA, USA). p < 0.05 was considered significant.

3. Results

3.1 \( \text{H}_2\text{O}_2 \) enhanced the spontaneous phasic contractions of isolated human-bladder strips in a concentration-dependent manner

We found that 112 of 186 (60%) of human-bladder strips displayed phasic SBCs during an equilibration period of 30–40 min. Nevertheless, SBCs were evoked with carbachol (1μM). Carbachol (1μM) treatment increased the baseline tension by 1.2 ± 0.3 g (n = 62 strips).

Application of \( \text{H}_2\text{O}_2 \) (from 1μM to 10mM) produced a concentration-dependent increase in the SBCs of human-bladder strips (Fig. 1A). This enhancement manifested mainly as an increase in the baseline tone and amplitude of phasic contractions, and there was no change in the frequency of phasic contractions (Fig.1A). The enhancement could last >20 min. Therefore, the response to \( \text{H}_2\text{O}_2 \) was evaluated with the percent increase in AUC as well as the net change of Δg from the control. A significant desensitization effect was observed at higher doses (100μM) of \( \text{H}_2\text{O}_2 \) (Supplementary Fig. 1). Thus, noncumulative concentration–response curves of \( \text{H}_2\text{O}_2 \) were established (Fig. 1C) and revealed an EC_{50} of 175.8μM and 40.3μM for AUC and Δg, respectively (Fig. 1C). We did not see the inhibitory effects of \( \text{H}_2\text{O}_2 \) during a 30-min observation period for any concentration. There was no significant difference in the amplitude of the \( \text{H}_2\text{O}_2 \) (100μM)-induced increase in Δg between carbachol-treated (Δg = 0.85 ± 0.12g/g, n = 12) and untreated strips (Δg = 0.78 ± 0.23g/g, n = 7).

3.2 Activation of TRPA1 channels is involved in \( \text{H}_2\text{O}_2 \)-induced enhancement effects

Studies have shown that \( \text{H}_2\text{O}_2 \) can target TRPA1 channels[28, 32, 36]. To determine the involvement of TRPA1 activation in \( \text{H}_2\text{O}_2 \)-induced effects, HC-030031 (30μM), a potent and selective inhibitor of TRPA1[37], was applied 5–10 min before and during \( \text{H}_2\text{O}_2 \) application. HC-030031 applied alone had no effects on contractile activity. \( \text{H}_2\text{O}_2 \)-induced enhancement was attenuated significantly in the presence of HC-030031 (Fig. 1B), and the concentration–response curve of AUC and Δg was shifted to the right (Fig. 1C). In the presence of HC-030031, EC_{50} was 1.29 mM and 743.9μM for AUC and Δg, respectively, which was much larger than that of the control. The maximum response (E_{max}) was reduced by 37.8% and 15.3% for AUC and Δg, respectively, in the presence of HC-030031. These observations suggested that TRPA1 activation was involved in \( \text{H}_2\text{O}_2 \)-induced effects.

Similar to \( \text{H}_2\text{O}_2 \), AITC (10μM, 30μM, 100μM, 300μM, 1mM), a specific agonist of TRPA1, also produced a concentration-dependent increase in the SBCs of human-bladder strips (Fig. 2A). The concentration–response curve of AITC revealed an EC_{50} of 195.3μM for AUC (Fig. 2B). However, a reliable EC_{50} for Δg could not be obtained because saturation was not reached even at 1 mM of AITC. The enhancement effect of AITC (300μM) could be blocked by HC-030031 (30μM) (Fig. 2C). The AUC and Δg were reduced by 73% and 62.4%, respectively, in the presence of HC-030031 (Fig. 2D). Because \( \text{H}_2\text{O}_2 \) at 100μM and AITC at 300μM produced a prominent increase in the SBCs of human-bladder strips, they were used as the test concentrations in subsequent experiments.

3.3 TRPA1 expressed on sensory nerves is involved in \( \text{H}_2\text{O}_2 \)-induced enhancement effects

Nicholas et al showed that \( \text{H}_2\text{O}_2 \) increased the firing activity of bladder sensory afferents mainly by activating TRPA1 channels[28]. It is accepted that sensory afferents regulate SBCs by releasing neurotransmitter CGRP or SP, a process termed “efferent actions of sensory afferents” [23, 24, 26, 38, 39]. To examine the involvement
of TRPA1 on bladder sensory nerves in H$_2$O$_2$-induced effects, bladder strips were treated with a high dose of capsaicin (10μM) 10–15 min before and during H$_2$O$_2$ to desensitize bladder sensory nerves. Capsaicin applied alone did not impact the contractile activity. Desensitizing sensory afferents with capsaicin reduced the H$_2$O$_2$ (100μM)-induced increase in AUC and Δg significantly by 68.1% and 71.5%, respectively (p<0.05) (Fig. 3A and B). After desensitization of sensory nerves, the AITC (300μM)-induced increase in AUC and Δg was reduced by 71.1% and 73.7%, respectively (p<0.05) (Fig. 3C and D).

To further confirm the involvement of activation of sensory afferents, TTX (1μM) was applied to block nerve firing. TTX applied alone did not impact the contractile activity. However, application of TTX (1μM) reduced the H$_2$O$_2$ (100μM)-induced increase in the AUC and Δg significantly by 61.3% and 72.9%, respectively (Fig. 3A and B). Application of TTX (1μM) reduced AITC (300μM)-induced increase in AUC and Δg significantly by 73.5% and 70.9%, respectively (Fig. 3C and D).

Immunofluorescence experiments revealed TRPA1 channels on sensory afferents located in sub-urothelial and intramuscular layers. These sensory afferents also expressed SP (Fig. 4A and B).

### 3.4 Release of SP and PGE2 contribute to H$_2$O$_2$-induced enhancement effects

Tachykinins are neuropeptides present in the capsaicin-sensitive primary afferents of the urinary bladder of various species (including humans). Release of these peptides in the periphery produced prominent effects on the SBCs of isolated bladder strips[3]. In agreement with this report, application of SP (500nM) increased the SBCs of human-bladder strips significantly (Fig. 5A), and the enhancing effect of SP could be blocked by a specific antagonist of NK2Rs: SR 48968 (10μM). AUC and Δg were reduced by 84.5% and 64.3%, respectively (Fig. 5A, B). NK2R is the main subtype of neurokinin receptor that mediates the effects of neurokinins in the human bladder[40-42]. SR 48968 applied alone did not impact the contractile activity. As expected, the NK2R antagonist SR 48968 (10μM) reduced the H$_2$O$_2$ (100μM)-induced increase in SBCs significantly (Fig. 5C). AUC and Δg were reduced by 74.1% and 49.1%, respectively (Fig. 5C), which suggested that SP release contributed to the enhancement effects of H$_2$O$_2$.

PGE2 released from sensory afferents, urothelial cells, or the detrusor has been shown have important modulatory effects on SBCs in vitro [43] and voiding behavior in vivo [44]. In agreement with those reports, application of PGE2 (1μM) evoked a significant increase in the SBCs of human-bladder strips (Fig. 6A). Pretreatment (15 min before H$_2$O$_2$) with indomethacin (10μM) to block the synthesis and release of PGE2 led to significant attenuation of H$_2$O$_2$ (100μM)-induced effects, with AUC and Δg being reduced by 65.9% and 35.9%, respectively (Fig. 6B and C). Indomethacin (10μM) applied alone did not affect contractile activity. This observation indicated that PGE2 release contributed to the enhancing effects of H$_2$O$_2$.

### 4. Discussion

Excessive ROS can lead to DO. To investigate the underlying mechanisms, the effects of H$_2$O$_2$ on SBCs of isolated human-bladder strips were investigated in the present study. To our knowledge, this is the first study to examine the mechanisms in human bladder. We found that H$_2$O$_2$(1μM to 10mM) concentration-dependently increased the SBCs of human-bladder strips. These enhancing effects could be mimicked by an agonist of TRPA1 channels, and could be blocked with an antagonist of TRPA1 channels. H$_2$O$_2$ induced enhancing effects could be attenuated by desensitizing sensory afferents with capsaicin, blocking nerve firing with TTX, blocking neurokinin effects with NK2 receptor antagonist and blocking PGE2 synthesis with indomethacin, respectively. Our results suggested activation of TRPA1 channels on bladder sensory afferents, then causing the release of SP or PGE2 from nerve terminals is one of the underling mechanisms for ROS leading to DO.

In the present study, low concentration of H$_2$O$_2$ produced similar stimulatory effects on SBCs in isolated strips of human bladder and rat bladder [17, 19]. H$_2$O$_2$ can act on several cell targets or pathways to induce enhanced contraction of the bladder. H$_2$O$_2$ could target the detrusor muscle directly to enhance SBCs via activation of cyclooxygenase or rho-kinase pathways which, in turn, increase extracellular Ca$^{2+}$ influx into the detrusor[17, 19]. H$_2$O$_2$ may target urothelium cells to evoke DO via ATP release, as suggested
by Stephany et al. [45]. Activation of bladder sensory afferents has been shown to be another important mechanism for H<sub>2</sub>O2-induced DO [15, 46].

Activation of sensory afferents may work through two mechanisms to enhance bladder contractile activity: (i) by increasing the spinal reflex or (ii) by increasing neurotransmitter release in the periphery via a local axon reflex (as suggested by Maggie and Gillespie) [25, 26]. Our most important finding was that peripheral activation of bladder sensory afferents and SP release had crucial roles in H<sub>2</sub>O2-induced effects in the human bladder, which support (ii). This conclusion was based on our two results. First, desensitization of bladder sensory afferents with capsaicin or blockade of nerve firing with TTX attenuated the effects of H<sub>2</sub>O2 significantly. Second, the H<sub>2</sub>O2-induced enhancement of SBCs was mimicked with SP and reduced significantly by antagonists of NK2Rs, which suggested SP release. However, our results did not deny the importance of the spinal reflex pathway evoked by sensory-afferent activation in vivo [15] and did not exclude the other possible underlying mechanisms mentioned above.

TRPA1 channels are expressed predominantly in the sensory-afferent nerve endings of the bladder [27]. One study in guinea pigs showed that H<sub>2</sub>O2 evoked long-lasting firing of capsaicin-sensitive high-threshold bladder afferents by activating TRPA1 [28]. We also found that activation of TRPA1 channels on bladder sensory afferents mediated the enhancing effects of H<sub>2</sub>O2 in the human bladder. The supporting evidence was: (1) a specific antagonist of TRPA1, HC-030031, attenuated the H<sub>2</sub>O2-induced increase in SBCs significantly (Fig. 1); (2) the H<sub>2</sub>O2-induced increase in SBCs was mimicked by TRPA1 agonists (Fig. 2); (3) TRPA1 channels were expressed on bladder sensory afferents (Fig. 4); (4) studies have shown that H<sub>2</sub>O2 mainly targets TRPA1 in sensory afferents [28, 32]. In addition to sensory afferents, TRPA1 has been shown to be localized in the urothelial cells of the urinary bladder [47], but urothelial expression of TRPA1 is species-specific [28]. TRPA1 in the bladder urothelium might be involved in sensory transduction in the bladder and OAB induction by BOO [47, 48]. Our immunohistology study also revealed TRPA1 expression in human urothelial cells (Fig. 4A) but Ca<sup>2+</sup> imaging studies did not reveal functional expression of TRPA1 in disassociated human urothelial cells (data not shown), which indicated a low possibility for urothelial TRPA1 contribution to the effects of H<sub>2</sub>O2. However, we could not exclude the involvement of TRPA1 on sub-urothelial interstitial cells (ICs) given the important modulatory role of these ICs in SBCs [20] and functional expression of TRPA1 in human sub-urothelial ICs revealed by our previous study [49]. However, the significant reduction of H<sub>2</sub>O2-induced effects by desensitization of sensory afferents with capsaicin and blockade of nerve firing with TTX may suggest that the effect of TRPA1 on sensory afferents has a dominant role.

SP is a neuropeptide present in the capsaicin-sensitive primary afferent nerves of the urinary bladder. SP release in the periphery produced enhancing effects on detrusor contractions [3]. In accordance with studies in the human bladder [40, 41], SP produced prominent enhancement in SBCs (Fig. 5A). Furthermore, an antagonist of NK2Rs (the dominant receptor subtypes mediating the effects of neurokinins in the human bladder) [40-42] attenuated the H<sub>2</sub>O2-induced increase in SBCs significantly (Fig. 5C and D), which suggested the involvement of SP and NK2Rs in the effects of H<sub>2</sub>O2. This result is in accordance with studies showing SP release contribute to increased SBCs induced by a TRPA1 agonist in isolated rat bladder [31].

PGE2 is an important modulator of bladder function and micturition. Studies have shown PGE2 release contributed to TRPA1 activation-induced increase in SBCs in the bladder of rats and guinea pigs [29, 31, 50]. Our data also indicated the involvement of PGE2 in the H<sub>2</sub>O2-induced enhancement of SBCs in human-bladder strips. First, PGE2 reproduced similar enhancing effects to those elicited by H<sub>2</sub>O2. Second, blockade of the synthesis and release of PGE2 with indomethacin attenuated H<sub>2</sub>O2 (100μM)-enhancing effects significantly (Fig. 6A-B). The role of PGE2 in H<sub>2</sub>O2-induced effects may further prove sensory-afferent activation by H<sub>2</sub>O2. However, unlike SP (which is released mainly from sensory nerve terminals), PGE2 can be synthesized and released by cells in the urothelium or detrusor in addition to sensory nerves. An interaction between SP and PGE2 has been proposed that release of SP in response to TRPA1 activation on sensory afferents could stimulate PGE2 production in nerve endings via an autocrine process [50], this idea may support our proposal.

Our study had three main limitations. First, we did not provide direct evidence for the release of SP.
and PGE2: only pharmacological blockade experiments were undertaken. Second, we did not test the involvement of other TRP channels (e.g., TRPV1), because literature indicates that TRPA1 is the dominant channel responsive to $H_2O_2$[28]. Third, we did not test the role of TRPA1 on ICs in $H_2O_2$-induced effects.

5. Conclusions

Our study provides evidence that $H_2O_2$, at a concentration range associated with inflammation and ischemia–reperfusion[28], enhanced SBCs of human-bladder. Activation of TRPA1 channels on peripheral sensory nerves and then the release of SP or PGE2 were important mechanism for $H_2O_2$ induced enhancing effects. Our study provides new insights for the mechanisms underlying ROS leading to OAB. Targeting TRPA1 channels might be the promising strategy for clinical treatment of OAB in conditions associated with excessive production of ROS.

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Data availability

Data will be made available on request.

Conflict of interest

None of the contributing authors have any conflicts of interest to disclose.

Figure legends

Figure 1. $H_2O_2$ concentration-dependently enhanced the spontaneous bladder contractions (SBCs) of isolated human-bladder strips, which could be inhibited by an antagonist of TRPA1: HC-030031. (A) Representative traces showing $H_2O_2$ (1μM–10 mM) enhanced SBCs. Enhancement manifested mainly as an increase in the baseline tone and amplitude, but not the frequency, of phasic contractions. Each strip was treated with only one concentration of $H_2O_2$. (B) Representative traces showing $H_2O_2$-induced increase in SBCs was attenuated in the presence of an antagonist of TRPA1: HC-030031 (HC03; 30 μM). HC03 was applied 5-10 min before and during application of $H_2O_2$. (C) Noncumulative concentration–response curves of $H_2O_2$ in the absence and presence of HC03. AUC and baseline tension were measured at 15-min intervals immediately before and after $H_2O_2$ treatment. $H_2O_2$-induced effects were evaluated by the percent increase in AUC and net increase in baseline tension ($Δg = after minus before$). $Δg$ was normalized further with the wet weight of the strip ($Δg/g$). Solid lines represent the fitted curves using the Hill equation, from which were obtained an EC$_{50}$ of 175.8 μM (HC03 + $H_2O_2$) for AUC; 40.3 μM (HC03 + $H_2O_2$) for $Δg$. *p < 0.05, **p < 0.01, ***p < 0.001 by one-sample t-test. Data are the mean ± SEM. Data from n = 4–6 strips were averaged for each concentration.

Figure 2. Activation of TRPA1 produced similar enhancing effects to $H_2O_2$ in isolated human-bladder strips. (A and B) Representative traces (A) and summary data (B) showing that AITC (10–1000μM) increased SBCs in a concentration-dependent manner. The concentration–response curves of AITC-induced increase in AUC (top) gave an EC$_{50}$ of 195.3μM for AUC, and a reliable EC$_{50}$ for $Δg$ (below) could not be obtained because saturation was not reached even at 1 mM of AITC. (C and D) Representative traces (C) and summary data (D) showing AITC (300μM)-induced increase in SBCs was blocked partially by the TRPA1 antagonist HC-030031 (HC03; 30μM). HC03 was applied 5-10 min before and during AITC application. n: number of the bladder strips; N: number of human samples. ***p < 0.001.

Figure 3. Desensitization of bladder sensory afferents with capsaicin or blockade of sodium channels with TTX attenuated $H_2O_2$-induced enhancing effects. (A and B) Representative traces (A) and summary data (B) showing pretreatment with capsaicin (10μM) or TTX (1μM) reduced $H_2O_2$-induced increases in SBCs of human-bladder strips significantly. Capsaicin or TTX was applied 15 min
before and during H2O2 (100μM) administration. (C and D) Representative traces (C) and summary data (D) showing pretreatment with capsaicin (10μM) or TTX (1μM) reduced AITC (100μM)-induced increases in SBCs of human-bladder strips significantly. n: number of the bladder strips; N: number of human samples. ***p < 0.001.

Figure 4. TRPA1 channels are expressed on the sensory afferents located in sub-urothelial and detrusor layer of the human bladder. Immunofluorescence for SP (red, 400× magnification) and TRPA1 (green, 400× magnification) in sub-urothelial (A) and intermuscular (B) nerve terminals and their co-localization (yellow, 400× magnification).

Figure 5. A selective antagonist of NK2 receptors attenuated H2O2-induced enhancing effects. (A and B) Representative traces (A) and summary data (B) showing SP (500nM) administration induced a prominent increase in SBCs of human-bladder strips, which were attenuated by pretreatment with a selective antagonist of NK2 receptors: SR 48968 (10μM). SR 48968 was applied 5 min before and during H2O2 (100μM) administration. (C and D) Representative traces (C) and summary data (D) showing pretreatment with SR 48968 attenuated H2O2-induced increase in SBCs. n: number of bladder strips, N: number of human samples. ***p < 0.001.

Figure 6. Blockade of PGE2 synthesis with indomethacin attenuated H2O2-induced enhancing effects. (A-B) Representative traces showing PGE2 (1μM) administration induced enhancement in SBCs (A), and that pretreatment with indomethacin attenuated H2O2-induced increases in SBCs (B). Indomethacin was applied 10–15 min before and during H2O2 (100μM) administration. (C) Summary data showing pretreatment with indomethacin reduced H2O2 (100μM)-induced increases in AUC and Δg significantly. *p < 0.05.

Supplementary Figure 1. Desensitization effect of repeat application of H2O2. (A) Typical traces showing that, on the same bladder strip, second-time application of H2O2 evoked a lower response compared with first-time application. (B) Summary data from five strips. **p < 0.01.

References:


Fig. 6

A

PGE₂

1 μM

0.4 μM

B

H₂O₂

100 μM

INDO+H₂O₂

100 μM

C

% increase in AUC

mM, N=3

mM, N=2

Tensions increased (g/g)

mM, N=3

mM, N=2

This a preprint and has not been peer reviewed. Data may be preliminary.