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The transient receptor potential A1 ion channel (TRPA1) modifies in vivo autonomous ureter peristalsis in rats

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Abstract

Aims: The current study aimed to explore the expression of transient receptor potential A1 ion channels (TRPA1) in the rat ureter and to assess if TRPA1-active compounds modulate ureter function.

Methods: The expression of TRPA1 in rat ureter tissue was studied by immunofluorescence. The TRPA1 distribution was compared to calcitonin gene-related peptide (CGRP), α-actin (SMA1), anoctamin-1 (ANO1), and c-kit. For in vivo analyses, a catheter was implanted in the right ureter of 50 rats. Ureter peristalsis and pressures were continuously recorded by a data acquisition set-up during intraluminal infusion of saline (baseline), saline plus protamine sulfate (PS; to disrupt the urothelium), saline plus PS with hydrogen sulfide (NaHS) or cinnamaldehyde (CA). Comparisons were made between rats treated systemically with vehicle or a TRPA1-antagonist (HC030031).

Results: TRPA1-immunoreactive nerves co-expressed CGRP and were mainly located in the suburothelial region of the ureter. Immunoreactivity for TRPA1 was also encountered in c-kit-positive but ANO1-negative cells of the ureter suburothelium and wall.

In vivo, HC030031-treated rats had elevated baseline peristaltic frequency ($p < 0.05$) and higher intraluminal pressures ($p < 0.01$). PS increased the frequency of ureter peristalsis versus baseline in vehicle-treated rats ($p < 0.001$) but not in HC030031-treated rats. CA ($p < 0.001$) and NaHS ($p < 0.001$) decreased ureter peristalsis. This was counteracted by HC030031 ($p < 0.05$ and $p < 0.01$).

Conclusions: In rats, TRPA1 is expressed on cellular structures considered of importance for peristaltic and mechanoafferent functions of the ureter. Functional data indicate that TRPA1-mediated signals regulate ureter peristalsis. This effect was pronounced after mucosal disruption and suggests a role for TRPA1 in ureter pathologies involving urothelial damage.

KEYWORDS

ankyrin 1, cinnamaldehyde, H_2S, interstitial cell, nerve, pacing

1 | INTRODUCTION

The ureters ensure unidirectional transport of urine from the kidneys to the urinary bladder by peristalsis that is described to involve activities of pacemaker cells, mechanosensory signals, and electromechanical coupling of myogenic functions.[1-3] The interface between components of the ureter peristalsis system is not fully known. Electrical recordings during pyeloureteric motility have been correlated to peristalsis in various species and the pacing of such signals are proposed to arise from atypical smooth muscle cells and interstitial cells of Cajal-like cells (ICC-LC).[1,3,4] Plasticity in the expression or function of these cells are proposed to cause discordant regulation of ureter peristalsis in relation to vesicoureteral reflux, congenital ureteropelvic junction obstruction, and obstructive megaureter.[2,3]

Besides its barrier function, the mucosa of the urinary tract is a sensory unit that responds to mechanical or chemical provocation with local release of factors that regulate activities of other cell types that are involved in, for example, motor functions, cell turn-over, inflammation, or pain perception.[5] As such, pressure-dependent signals from the urothelium has been reported to modify the activity of sensory neurons of the perfused ureter, and capsaicin-dependent signals and tachykinins are reported to modify spontaneous contractile activities of upper urinary tract preparations.[3,6] Cellular structures within the ureter mucosa, that is, the urothelium, interstitial cells, and neurons, are express multimodal transient receptor potential ion channels (TRPs) that respond to chemical, mechanical, and thermal stimuli.[7,8] Information is scarce on the functional role of TRPs in normal ureter peristalsis or ureter disease. The ankyrin 1 subtype TRP (TRPA1) has been located to sensory neurons, and interstitial cells of the proximal human ureter, and TRPA1-mediated signals have been proposed involved in modulation of neuromuscular functions of the isolated human and pig ureters.[8,9] The TRPA1 is a cold- and mechanoreceptor that also acts as a sensor to irritants and bacterial lipopolysaccharides, is activated by hypoxia, and is described to be involved in neuroinflammatory responses of viscera.[8,10] If TRPA1-related transmission is involved in the regulation of ureter peristalsis has, to the best of our knowledge, not been explored. Therefore, the aim of this study was to investigate the effects by various TRPA1-active compounds on ureter peristalsis in vivo in a rat model. In addition, we assessed the expression of TRPA1 in the rat ureter in comparison to our previous findings of the human ureter.[8]

2 | MATERIALS AND METHODS

2.1 | Ethical considerations

All experiments were approved by the Institutional Animal Ethics Care and Use Committees of the Lund and Linköping Universities, Sweden, and San Raffaele Scientific Institute, Milan, Italy. Experiments were carried out in accordance with the ARRIVE Guidelines.[11]

2.2 | Animals

Fifty male Sprague–Dawley rats (250–300 g; Charles River) were used. Animals were maintained under standard laboratory conditions with a 12:12 h light:dark cycle, free access to food, water, and enrichments. Isoflurane (alveolar concentration of 5% for induction and 2% for maintenance) was used as anesthesia. After experiments, rats were killed during anesthesia by carbon dioxide asphyxia. Ureters were harvested for immunofluorescence investigations from six naive separate male rats.

2.3 | Immunofluorescence

Ureter specimens were processed for immunofluorescence as previously described.[12] Primary antibodies: rabbit anti-TRPA1 (1:500; Alomone Labs), guinea pig anti-calcitonin gene-related peptide (CGRP; a marker for sensory nerves, 1:750; Euro-Diagnostica), mouse anti-smooth muscle α-actin (SMA; 1:1000; Abcam), goat anti-anoctamin-1 (ANO1; a marker for interstitial cells, 1:250; Santa Cruz), and a mouse anti-c-kit (a marker for interstitial cells, 1:250; Santa Cruz). Secondary antibodies: Alexa Fluor, Molecular Probes Inc. (1:800). Sections were analyzed using a laser fluorescence microscope (Olympus Corporation). Images were acquired using Viewfinder Lite version 2.0 (Pixera Corp.).

2.4 | In vivo experimental procedures

Animals were randomized to experimental groups (QuickCalcs; GraphPad Software Inc.). Briefly, during anesthesia, an abdominal incision was performed. A polyethylene catheter (PE-10; Clay-Adams) was introduced into the ureteropelvic junction of the left ureter.[13] The catheter was attached to a microsyringe pump (CMA 100; Carnegie Medicine AB) and connected to a pressure transducer (see Supplementary Information). Room temperature physiological saline was infused at a speed of 0.4 ml h^{-1} simulating normal urine production.[13] Ureteral

intraluminal pressure was recorded with an MP100 data acquisition system (Biopac Systems Inc.). The bladder was incised to avoid interference on ureter pressures from detrusor contractions.[13]

In pilot experiments (data not shown), the TRPA1 agonists did not effect ureter peristalsis or pressures when given intraluminally in naive rats. Therefore, after a baseline registration (20–30 min) with a saline infusion to achieve stable autonomous peristaltic ureter activity, an infusion with a protamine sulfate (PS)-containing (10 mg/ml) saline solution to disrupt the urothelial mucous barrier function was used.[12] After achieving new stable autonomous activities, ureters were infused with a saline solution containing the TRPA1-agonists cinnamaldehyde (CA) at 10 mM (1.32 mg/ml) or sodium hydrosulfide (NaHS - a donor of H2S) at 1 μM (0.056 μg/ml), 100 μM (5.6 μg/ml) or 10 mM (0.56 mg/ml). Before PS-treatment, some animals were given the TRPA1-antagonist HC030031 (0.7 mg/kg) or vehicle by intraperitoneal injection.

The following ureteral parameters were analyzed: frequency (contractions per minute) and amplitude (cmH_2O) of autonomous peristaltic pressure waves, minimum pressure (MinP; cmH_2O), maximum pressure (MaxP; cmH_2O) and the AUC per second (cmH_2O/s).

2.5 | Drugs and solutions

CA (Sigma-Aldrich) was dissolved in 50% ethanol, HC030031 (Sigma-Aldrich) in DMSO, and NaHS and PS (Sigma-Aldrich) in saline. HC030031 was further diluted in mineral oil and the other drugs were diluted in saline.

2.6 | Data analysis

Records were analyzed by separate investigators that were blinded to experimental protocols. Values are given as mean ± *SEM*. Student Newman–Keuls analysis of variance for repeated measures and Student's *t* test was used for statistical analyses (SigmaPlot 11.0 software; Systat Software Inc.). All calculations are based on the number of individuals. Differences were considered significant when $p < 0.05$.

3 | RESULTS

3.1 | Immunohistochemistry

TRPA1 immunoreactivity was found in nerves and nerve terminals of the suburothelial region of the rat ureter (Figure 1). A majority of TRPA1-immunoreactive nerves also expressed immunoreactivity for CGRP (Figure 2). Intense TRPA1 immunoreactivity was also located in cells interspersed in the suburothelial region (Figures 1 and 2). Single TRPA1-positive cells were encountered in the muscular layer of the ureter wall (Figure 1). Co-stainings of sectioned rat ureter tissue with antibodies for TRPA1 and ANO1 disclosed no overlap of immunoreactivities (Figure 2) In contrast, TRPA1-immunoreactive cells also expressed c-kit-immunoreactivity (Figure 2).

3.2 | In vivo ureteral peristalsis at baseline and after PS (Table 1A)

One animal died during surgical procedures and two animals were excluded due to catheter problems. In remaining animals, all ureters exhibited autonomous spontaneous peristaltic activity recorded as regular intraluminal pressure waves (Figures 3 and 4). At baseline, before infusion of PS in ureters of rats unexposed to any drugs, 4.0 ± 0.2 ($n = 29$) peristaltic contractions per minute were recorded. Minimum and maximal pressures amounted to 33.0 ± 2.7 and 41.7 ± 2.8 cmH_2O, respectively. The amplitude of the pressure waves was 8.7 ± 0.7 cmH_2O and the AUC/second was 35.7 ± 2.8 cmH_2O/s. After the intraluminal infusion of PS, the frequency of ureter autonomous activity increased (Figure 3) by 79 ± 13% ($p < 0.001$). MinP, MaxP, amplitude, or AUC/second were not changed.

3.3 | Effect of HC030031 on ureter in vivo peristalsis on baseline and after PS (Table 1A)

Rats administered HC030031 (0.7 mg/kg, ip, $n = 18$) had similar amplitude of ureter peristaltic pressure waves as controls. Compared to controls, HC30031-treated rats had an elevated baseline frequency ($p < 0.05$) and the MinP, MaxP, and AUC/second were higher ($p < 0.01$). After PS-treatment, no change from baseline in the frequency of ureter autonomous activity was observed in HC030031-treated rats (Figure 3) that exhibited 4.6 ± 0.3 (baseline) and 4.2 ± 0.3 (PS) peristaltic contractions/minute ($p < 0.001$ vs. control after PS). As for control rats, PS did not alter MinP, MaxP, amplitude, or AUC/second in HC030031-treated rats.

3.4 | Effect of CA with or without HC030031 on ureter in vivo peristalsis (Table 1B)

After pretreatment with PS, intraluminal infusion of CA (10 mM; $n = 7$; Figure 4) caused a 58 ± 4% decrease of

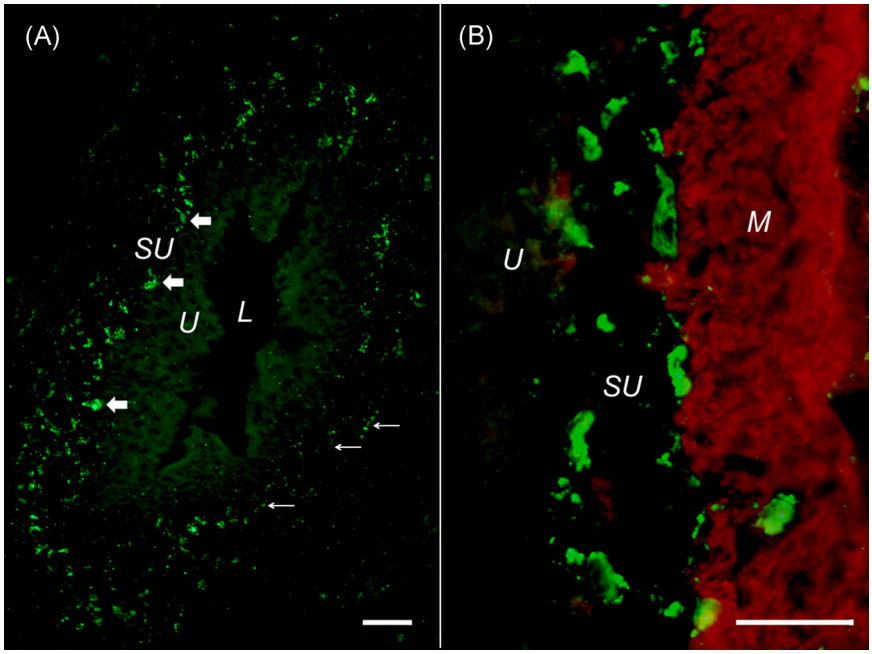

FIGURE 1 Immunofluorescence. (A) Transient receptor potential ion channel ankyrin 1 (TRPA1) in a transverse section of the rat mid-portion of the ureter. Intense Alexa Green stainings in cellular structures and nerve structures of the SU. Thick arrows highlight TRPA1 immunofluorescence in cellular structures. Thin arrows highlight varicose nerve terminals. Scale bar = 100 μM. (B) Mid-portion of a rat ureter. Double staining for transient receptor potential ion channel ankyrin 1 (TRPA1; Alexa Green) and smooth muscle α-actin (Alexa Red). Scale bar = 50 μM. L, lumen; M, muscular region; SU, suburothelial region; U, urothelium

peristaltic frequency ($p < 0.001$ vs. PS). CA had no effect on ureter pressures or amplitudes of contractions. In animals pretreated with HC030031 ($n = 6$; Figure 4), CA produced a 31 ± 10% reduction of the frequency of ureter peristalsis ($p < 0.05$ vs. CA without HC030031). Similar to vehicle, CA had no effect on ureter pressures or amplitudes in the presence of HC030031.

3.5 | Effect of NaHS with or without HC030031 on ureter in vivo peristalsis (Table 1C)

Intraluminal NaHS at 10 mM ($n = 9$), 100 μM ($n = 5$), or 1 μM ($n = 6$; Figure 4) caused 63 ± 5%, 57 ± 6%, and 59 ± 5% decrease of peristaltic frequency (all $p < 0.001$ vs. PS). At 1 μM, NaHS had no effect on pressures or amplitudes of spontaneous contractions. However, NaHS increased maximal pressure by 13 ± 4% and 27 ± 4% at 100 μM and 10 mM, respectively. At 10 mM of NaHS, the amplitude of autonomous contractions was increased from 7.1 ± 0.6 to 10.5 ± 0.7 cmH_2O ($p < 0.05$ vs. PS) and the MinP increased from 25.7 ± 4.3 to 31.3 ± 5.2 cmH_2O ($p = 0.07$ vs. PS). Other parameters were not altered by NaHS at 100 μM or 10 mM.

In rats treated with NaHS (10 mM) and HC030031 ($n = 5$), the effects by NaHS on intraluminal ureter pressures that were observed in vehicle-treated rats were absent. In HC030031-treated rats, the inhibitory effect on ureter peristalsis by NaHS was reduced to 29 ± 4% ($p < 0.01$ vs. CA without HC030031). Similarly, in rats treated with NaHS (1 μM) and HC030031 ($n = 6$; Figure 4), the inhibitory effect on ureter peristalsis by NaHS was reduced to 17 ± 7% (not significant vs. CA without HC030031).

4 | DISCUSSION

This study provides novel information on TRPA1-mediated modulatory functions of rat ureter peristalsis in vivo, and also locates TRPA1 to putative components of the ureter peristalsis system.

Specifically, we describe TRPA1-immunofluorescence on suburothelial nerves that co-express the sensory marker CGRP. This corresponds to the distribution of TRPA1 in sensory neurons expressing CGRP or TRPV1 in the human ureter and harmonizes with the general understanding of how sensory systems are involved in mechanoafferent functions of the ureter are organized.[1,5,8]

We also report the expression of TRPA1 on SMA-negative cells of the wall of the rat ureter. The TRPA1-positive cells also contained immunofluorescence for c-kit. In the ureter, or in ureteropelvic tissue from

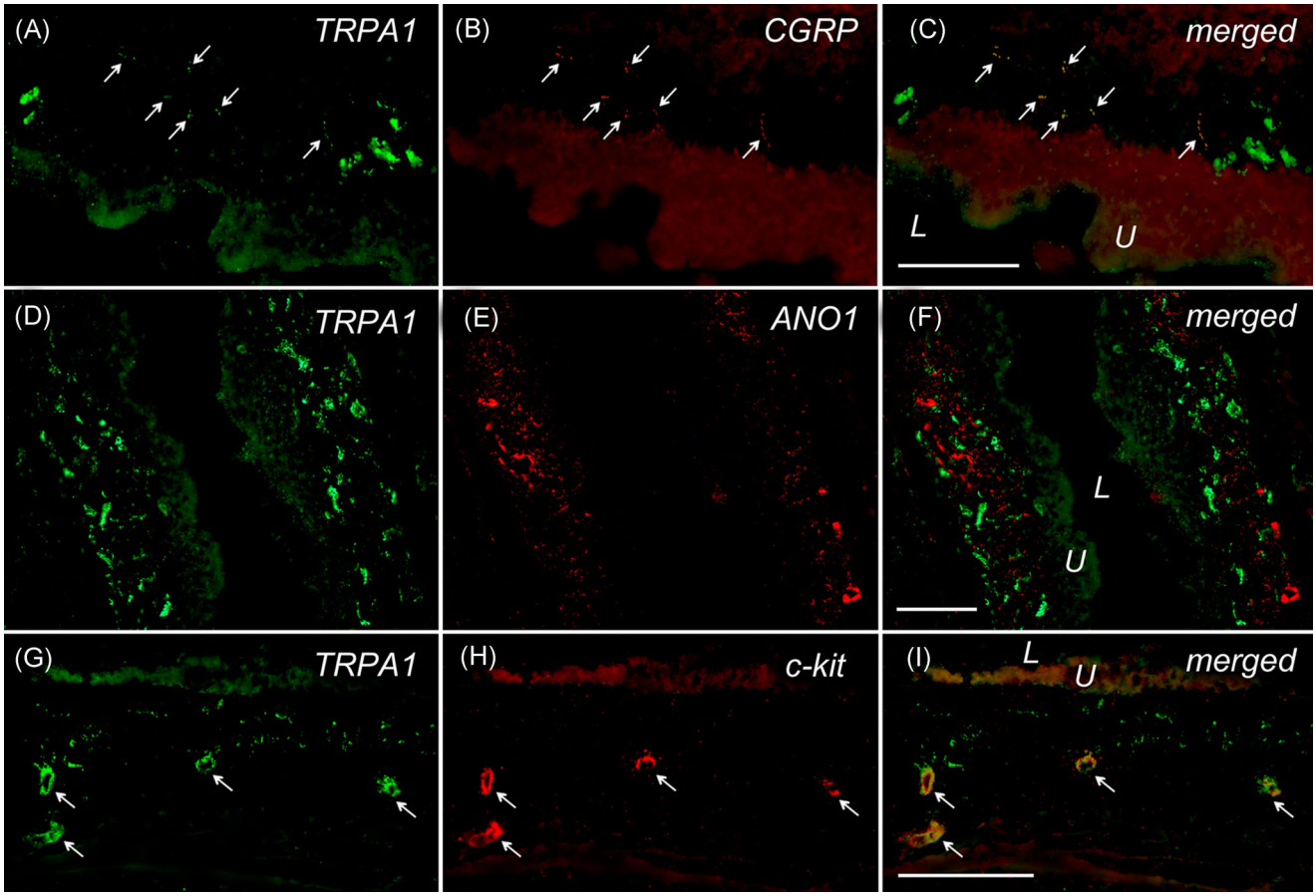

FIGURE 2 Immunofluorescence. Mid-portion of rat ureter. (A) Immunofluorescence reactivity for transient receptor potential ion channel ankyrin 1 (TRPA1) in suburothelial varicose nerve terminals (arrows) and six cells in the suburothelial region; Alexa Green. (B) The same section as in (A), depicting immunofluorescence for calcitonin gene-related peptide (CGRP) in the same varicose nerve terminals as in (A), arrows; Alexa Red. (C) Merged image of (A) and (B) depicting colocalization (yellow + arrows) of TRPA1- and CGRP-immunoreactivity in nerve varicosities of the rat ureter. (D) Immunofluorescence reactivity for TRPA1 in cells and nerves of the suburothelial region; Alexa Green. (E) The same section as in (D), immunofluorescence for anoctamin-1 (ANO1); Alexa Red. (F) Merged image of (D) and (E) depicting complete mismatch of TRPA1- (Alexa Green) and ANO1-immunofluorescence (Alexa Red) in nerves of the rat ureter. (G) Immunofluorescence reactivity for TRPA1 in varicose nerve terminals and four cells (arrows) of the suburothelial region; Alexa Green. (H) The same section as in (G), immunofluorescence for c-kit in the same four cells (arrows) as depicted in (G); Alexa Red. (I) Merged image of (G) and (H) depicting colocalization (yellow + arrows) of immunofluorescence for TRPA1 and c-kit in four cells. Scale bars = 50 μM. L, lumen; U, urothelium

various mammals, including humans, c-kit has previously been located to cells, that is, ICC-LCs, of similar morphology as ICCs in the gut. In the upper urinary tract, these c-kit-positive cells are distinct from smooth muscle cells and are considered required for pyeloureteric peristalsis.[2] Interestingly, an increased expression of c-kit in embryonic mice ureters was found to coincide with the development of unidirectional peristaltic contractions, and ureters treated with c-kit-neutralizing antibodies were unable to generate coordinated peristalsis.[14] Recently, it was shown that c-kit positive cells of the human ureter co-express hyperpolarization-activated cyclic nucleotide-gated channels that are important markers for pacing cells that exhibit autonomous electrical transients.[15] In addition to c-kit positive ICC-LCs as putative regulators of ureter peristalsis, atypical smooth muscle cells that exhibit spontaneous currents are proposed to trigger propagating action potentials in the smooth muscle layer.[1,4,16] In the rodent renopelvic region, SMA- and c-kit-negative interstitial cells have been reported to express the ANO1-channel that is suggested involved in pacemaker activity.[16] We also identified large numbers of ANO1-positive cells in the rat ureter but these cells were separately distributed from TRPA1-positive cells. Taken together, the current location of TRPA1 on sensory nerves and on ICC-LC of the rat ureter proposes that these structures could be targets for TRPA1-ligands that may have effects on mechano-afferent and peristaltic functions.

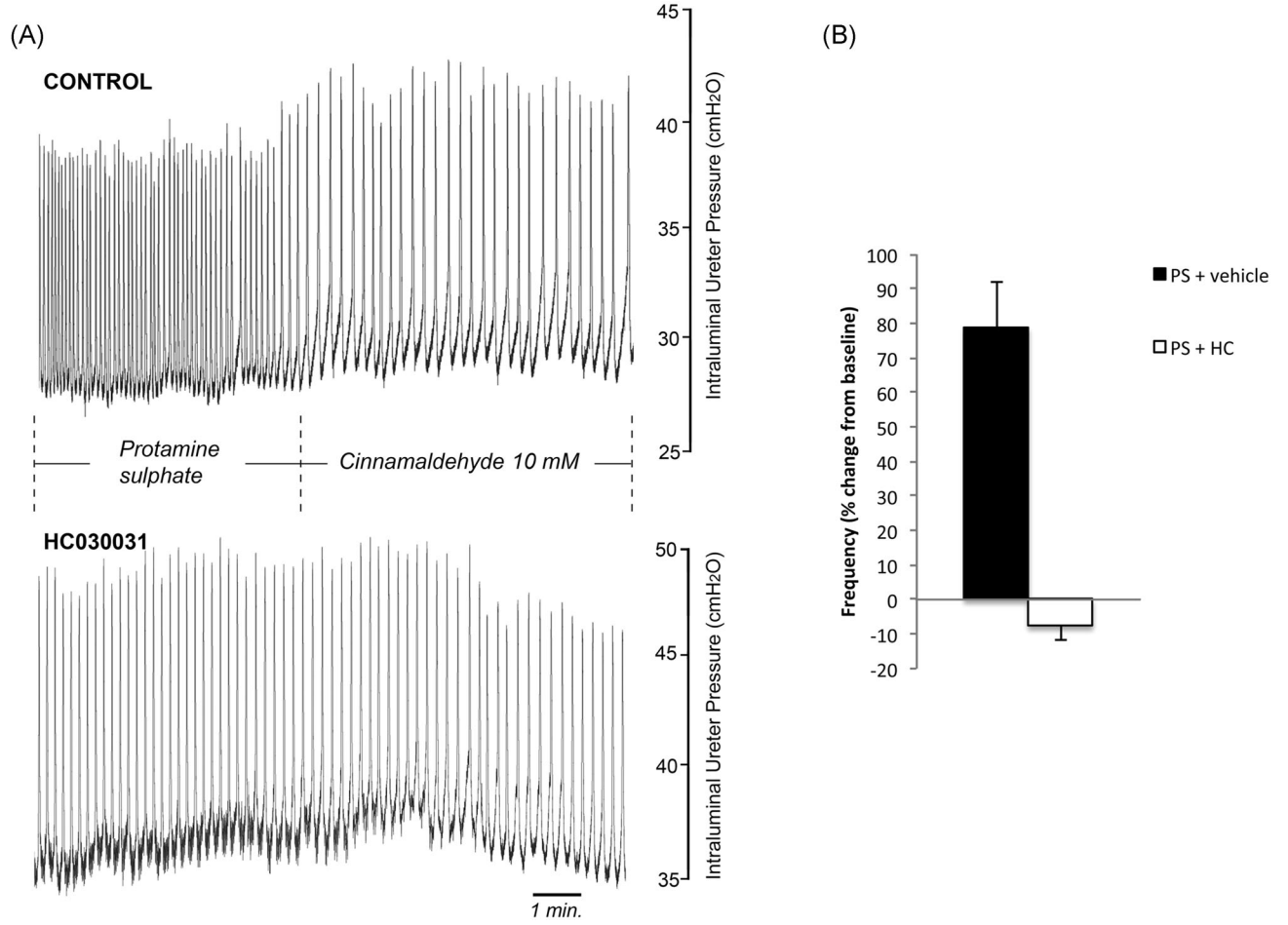

FIGURE 3 In vivo ureter pressure recordings. (A) Original tracing of in vivo ureteral peristalsis at baseline and after infusion of protamine sulfate (PS) in a control rat and in rat treated with intraperitoneal HC030031 (0.7 mg/kg). (B) Bar graph depicting change in ureteral peristaltic frequency of control rats treated with vehicle and in rat treated with intraperitoneal HC030031 (HC; 0.7 mg/kg) after infusion of PS. Values are given as mean ± standard error of the mean

We recorded autonomous peristaltic activity as regular recurrent pressure waves that were accompanied by visual antegrade propulsive movement of the ureter from proximal regions toward the urinary bladder. The baseline frequency of rat ureteric peristaltic activity was about four contractions per minute. This corresponds to our previous ureter recordings in vivo in rats and is similar to findings in rabbits, pigs, and humans.[13,17-19]

Interestingly, at baseline, HC030031-treated rats exhibited higher intraluminal pressures and AUC, and slightly elevated peristaltic frequency as compared to vehicle-treated rats, whereas no effects were observed on pressure-wave amplitudes. This may imply that TRPA1-mediated signals are involved in the regulation of ureter smooth muscle tonus and/or regulation of peristalsis in normal ureter physiology. Still, functional influences by TRPA1-active compounds on ureter peristalsis were more pronounced after the disruption of the urothelium.

To simulate urothelial damage (e.g., bacterial infection, stone disease, or inflammation), we administered PS intraluminally into ureters. This agent targets the mucus glycosaminoglycan (GAG) layer on the luminal side of the umbrella cells. The GAGs are hydrophilic and create a "water-trap" on the outside of umbrella cells that form an additional barrier between urine components and underlying structures.[20] During treatment with PS of vehicle-treated rats, we found that ureter peristaltic activity almost doubled in frequency. This finding implies that the urothelium of the ureter holds similar barrier functions as in the bladder and that when damaged, structures inside the mucosa are activated to modify ureter peristalsis. In the current setting, peristaltic stimuli could be exaggerated mechanical activation due to loss of the water-trap and/or absorption of luminal solutes, including various ions that may interact and depolarize nerves or other cells underneath the urothelium. Interestingly, rats treated systemically with the TRPA1

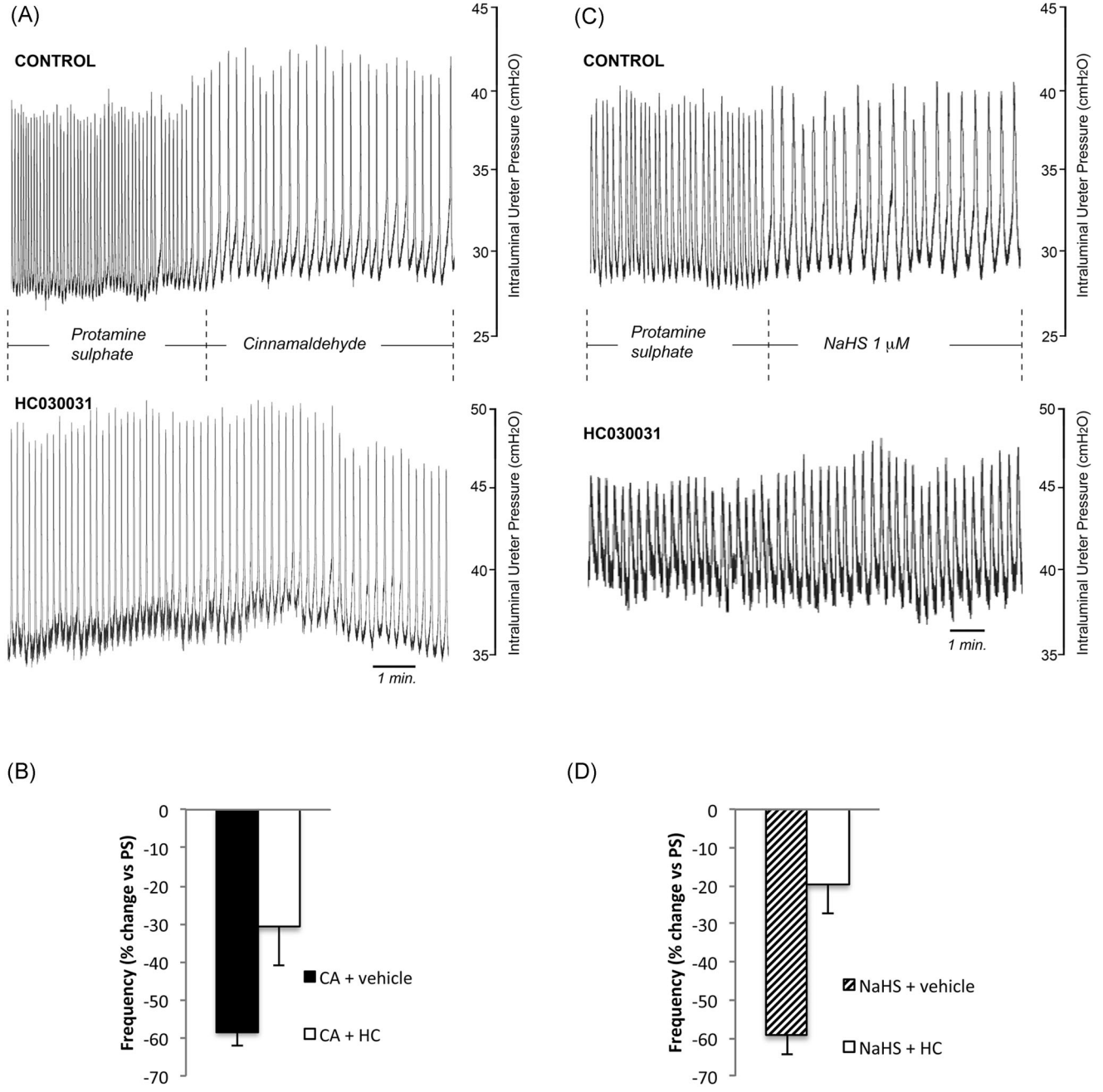

FIGURE 4 In vivo ureter pressure recordings. (A) Original tracing of the effect of intraluminal cinnamaldehyde (CA; 10 mM) on in vivo ureteral peristalsis during infusion of protamine sulfate (PS) in a control rat and in rat treated with intraperitoneal HC030031 (HC; 0.7 mg/kg). (B) Bar graph depicting the effect by CA (10 mM) on peristaltic frequency in rats treated with vehicle or HC (0.7 mg/kg). Values are given as mean ± standard error of the mean. (C) Original tracing of the effect of intraluminal sodium hydrosulfide (NaHS; 1 μM) on in vivo ureteral peristalsis during infusion of PS in a control rat and in rat treated with intraperitoneal HC (0.7 mg/kg). (D) Bar graph depicting the effect by NaHS (1 μM) on peristaltic frequency in rats treated with vehicle or HC (0.7 mg/kg). Values are given as mean ± standard error of the mean

antagonist HC030031 did not exhibit changes in peristaltic frequency to PS. Hence, TRPA1-mediated signals may, under conditions that involve urothelial damage, be involved in mechanoafferent functions related to the regulation of ureter peristalsis. In accordance to previous findings with intraperitoneal HC030031 in rodent models for nociception and allodynia, a main effect by the TRPA1-antagonist on sensory neurons and accompanying dorsal root ganglia supplying the ureter is assumed.[21]

Similar to urodynamic findings in rats, the TRPA1-agonists CA and NaHS did not produce functional in vivo effects when administered intraluminally into ureters of

TABLE 1 (A) Functional in vivo ureter parameters at baseline and after intraluminal infusion with protamine sulfate (10 mg/ml) in rats after intraperitoneal vehicle (saline, $n = 29$) or HC0300331 (0.7 mg/kg, $n = 18$)

	Frequency (Contraction min^{-1})	MaxP (cmH_2O)	MinP (cmH_2O)	Amplitude (cmH_2O)	AUC ($cmH_2O\ s^{-1}$)		Frequency (Contraction min^{-1})	MaxP (cmH_2O)	MinP (cmH_2O)	Amplitude (cmH_2O)	AUC ($cmH_2O\ s^{-1}$)
Vehicle baseline	4.0 ± 0.2	42.7 ± 2.8	33.0 ± 2.7	8.7 ± 0.7	35.7 ± 2.8	HC030031 baseline	4.6 ± 0.3°	54.4 ± 2.3°°	44.9 ± 2.1°°	9.5 ± 0.7	48.4 ± 2.2°°
Vehicle + PS	6.4 ± 0.4***	39.5 ± 3.3	31.8 ± 3.1	7.7 ± 0.7	35.1 ± 3.3	HC030031 + PS	4.2 ± 0.3°°°	54.3 ± 2.0°°	44.6 ± 2.0°°	9.6 ± 0.8°	47.4 ± 2.3°°

Note: Frequency = number of contractions (peristaltic pressure waves) per minute, MaxP = maximum intraluminal ureter pressure (cmH_2O), MinP = minimum intraluminal ureter pressure (cmH_2O), amplitude = MaxP−MinP (cmH_2O), and area under the curve (AUC; cmH_2O per second). Values are given as mean ± standrard error of the mean. ***$p < 0.001$ versus baseline (paired T test). °$p < 0.05$, °°$p < .01$, and °°°$p < 0.001$ between vehicle and HC030031 (unpaired T test).

(B) Functional in vivo ureter parameters after intraluminal infusion with protamine sulfate (10 mg/ml) and cinnamaladehyde (CA, 10 mM) in rats after intraperitoneal vehicle (saline, $n = 6$) or HC0300331 (0.7 mg/kg, $n = 5$)

	Frequency (Contraction min^{-1})	MaxP (cmH_2O)	MinP (cmH_2O)	Amplitude (cmH_2O)	AUC ($cmH_2O\ s^{-1}$)		Frequency (Contraction min^{-1})	MaxP (cmH_2O)	MinP (cmH_2O)	Amplitude (cmH_2O)	AUC ($cmH_2O\ s^{-1}$)
Vehicle + PS	7.5 ± 0.4	41.4 ± 6.3	32.6 ± 5.5	8.9 ± 1.9	36.0 ± 5.9	HC030031 + PS	3.5 ± 0.4°°	55.3 ± 4.0	43.3 ± 4.7	12.1 ± 1.9	44.5 ± 6.0
Vehicle + PS + CA	3.2 ± 0.4***	46.3 ± 6.2	35.6 ± 5.8	10.7 ± 1.3	39.6 ± 6.2	HC030031 + PS + CA	2.3 ± 0.2	56.8 ± 4.2	43.7 ± 4.7	13.2 ± 0.8	47.8 ± 4.7

Note: Frequency = number of contractions (peristaltic pressure waves) per minute, MaxP = maximum intraluminal ureter pressure (cmH_2O), MinP = minimum intraluminal ureter pressure (cmH_2O), amplitude = MaxP−MinP (cmH_2O), and area under the curve (AUC; cmH_2O per second). Values are given as mean ± standrard error of the mean. ***$p < 0.001$ versus baseline (paired T test). °°$p < 0.01$ between vehicle and HC030031 (ANOVA repeated measures).

(C) Functional in vivo ureter parameters after intraluminal infusion with protamine sulphate (10 mg/ml) and sodium hydrogensulphate (NaHS, 1 μM, $n = 6$; 100 μM, $n = 6$; or 10 mM, $n = 9$) in rats after intraperitoneal vehicle (saline)

	Frequency (Contraction min^{-1})	MaxP (cmH_2O)	MinP (cmH_2O)	Amplitude (cmH_2O)	AUC ($cmH_2O\ s^{-1}$)		Frequency (Contraction min^{-1})	MaxP (cmH_2O)	MinP (cmH_2O)	Amplitude (cmH_2O)	AUC ($cmH_2O\ s^{-1}$)
Vehicle + PS	6.3 ± 0.6	50.5 ± 5.0	43.0 ± 4.6	7.5 ± 1.1	46.8 ± 4.6	HC030031 baseline	4.6 ± 0.7	50.2 ± 2.0	41.3 ± 1.4	8.8 ± 1.0	45.6 ± 2.0
Vehicle + PS + NaHS 1 μM	2.4 ± 0.1***	50.8 ± 3.2	41.5 ± 3.1	9.2 ± 1.2	44.6 ± 3.2	HC030031 + PS + NaHS 1 μM	3.8 ± 0.8	49.3 ± 3.3	41.1 ± 2.3	8.1 ± 1.2	44.5 ± 2.8
Vehicle + PS	5.0 ± 0.6	47.9 ± 3.4	39.7 ± 3.1	8.3 ± 1.1	42.5 ± 4.2						
Vehicle + PS + NaHS 100 μM	2.2 ± 0.5***	54.1 ± 3.2*	41.4 ± 3.6	9.9 ± 1.5	44.4 ± 3.4						

TABLE 1 (Continued)

(C) Functional in vivo ureter parameters after intraluminal infusion with protamine sulphate (10 mg/ml) and sodium hydrogensulphate (NaHS, 1 μM, $n = 6$; 100 μM, $n = 6$; or 10 mM, $n = 9$) in rats after intraperitoneal vehicle (saline)									
	Frequency (Contraction min^{-1})	MaxP (cmH_2O)	MinP (cmH_2O)	Amplitude (cmH_2O)	AUC ($cmH_2O\,s^{-1}$)	Frequency (Contraction min^{-1})	MaxP (cmH_2O)	MinP (cmH_2O)	Amplitude (cmH_2O)	AUC ($cmH_2O\,s^{-1}$)
Vehicle + PS	6.6 ± 0.8	33.0 ± 4.5	25.7 ± 4.3	7.1 ± 0.6	28.9 ± 4.6	HC030031 + PS	4.8 ± 0.3	53.1 ± 4.3°	45.3 ± 3.4°	7.9 ± 1.1	48.6 ± 3.8°
Vehicle + PS + NaHS 10 mM	2.3 ± 0.3***	41.7 ± 5.3*	31.3 ± 5.2	10.5 ± 0.7*	34.4 ± 5.3	HC030031 + PS + NaHS 10 mM	3.5 ± 0.4*,°	54.6 ± 4.5	50.8 ± 5.4	8.9 ± 1.0	50.9 ± 4.0

Note: Effects by NaHS 1 μM or 10 mM were also studied after intraperitoneal HC0300331 (0.7 mg/kg). Frequency = number of contractions (peristaltic pressure waves) per minute, MaxP = maximum intraluminal ureter pressure (cmH_2O), MinP = minimum intraluminal ureter pressure (cmH_2O), amplitude = MaxP–MinP (cmH_2O), and area under the curve (AUC; cmH_2O per second). Values are given as mean ± standrard error of the mean. ***$p < 0.001$ versus baseline (paired T test). °$p < 0.05$ between vehicle and HC030031 (ANOVA repeated measures).

naive rats with an intact urothelium.[12] After inducing urothelial damage with PS, both CA and NaHS caused decreases in the peristaltic frequency of vehicle-treated rats. This supports an important role for the luminal urothelial mucus layer for the integrity of functions of the upper urinary tract. The recorded peristaltic effects by the TRPA1 agonists in PS-treated rats may be related to combined activities by the compounds on local neurotransmission and at interstitial cells of the ureter submucosa. Reports on TRPA1-mediated signals on pacemaker cell activities are scarce. However, NaHS has been shown to have direct inhibitory effects on ICCs from the mouse small intestine, and these findings were concluded related to decreases in gastrointestinal motility.[22] Binding of ligands to receptors of sensory nerves also evoke local efferent functions. Hence, acting as indirect "second messengers," for example, peptides or tachykinins, released from sensory nerve terminals in response to ligand–TRPA1 receptor interaction, maybe the actual effectors of a local response. In this context, it has been shown that NaHS induces TRPA1-mediated release of CGRP that has been shown to reduce the activity of pyeloureteric autorythmicity.[1,23] Another link between afferent signals and TRPA1 can be found in the properties of the local anesthetic lidocaine. Lidocaine has been reported to desensitize TRPA1-mediated signals, and upon administration in ureters, lidocaine reduces the peristaltic frequency in human subjects.[17,24] It may be speculated that the currently used TRPA1-agonists induce some form of desensitization of ureter mucosal afferents. TRPA1-mediated modulation of autonomic motor neurotransmission may also have contributed to our findings. TRPA1-agonists have been linked to inhibitory nerve-mediated responses of the pig and human ureter.[8,9] In agreement, TRPA1-mediated neuromodulation is also reported from other parts of the urinary tract.[25]

In PS-treated rats that were given HC030031 systemically, the inhibitory effect on peristaltic frequency by intraluminal administration of CA or NaHS was reduced by 20%–40% compared to vehicle-treated rats. Hence, systemic administration of a TRPA1 antagonist reduced effects by local administration of TRPA1-agonists on ureter peristaltic activity. These findings further evidence a role for this receptor in regulation of ureter peristaltic function under conditions that involve urothelial dysfunctions. We cannot exclude that intraperitoneal HC030031 may have effects in the central nervous system but believe that the agent putatively primarily inhibits sensory neurons that supply the ureter. This may explain why the local application of agonists still can produce additional effects on peristalsis and that this effect presumably occurs via actions on other mucosal pacing

structures that are more easily accessible after urothelial disruption. In support of this, HC030031- and PS-treated rats did not exhibit increased peristaltic frequency as compared to vehicle- and PS-treated rats, and as sequential local application of TRPA1-agonists in the ureters of HC030031- and PS-treated rats caused less effects on ureter peristalsis as compared to vehicle- and PS-treated rats. TRPA1-mediated depressing effect on stimuli-provoked increase of ureteric peristalsis may function to promote more efficient propulsion of ureter contents to evacuate noxious agents from the upper urinary tract.

Whereas intraluminal CA did not have any effects on intraureteric pressures or amplitudes, NaHS caused HC030031-sensitive increases in MaxP and amplitudes at higher concentrations. This descrepancy may be related to differences in the physicochemical properties of the compounds that may influence tissue penetrance. CA is a larger aromatic aldehyde, and NaHS is a donor of H2S, a small gaseous molecule, that in turn interacts with TRPA1. The observed effects on ureteric pressures by NaHS suggest that TRPA1-mediated effects also may modify the tonus of the ureter.

In summary, we conclude that TRPA1 in the rat ureter, similar to the human ureter, is expressed on cellular structures that are considered of importance for peristaltic and mechanoafferent functions of the ureter. We present functional data in vivo in rats that link TRPA1-mediated signals to the regulation of ureter peristalsis. These effects were pronounced in conditions when the urothelial barrier of the ureter was compromised and suggests a role for TRPA1 in ureter pathologies involving urothelial damage.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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