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# The enhancing effect of 5-HT on phasic contractions of human isolated distal ureter and possible mediating mechanisms

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**Abstract**

Abstract Background and purpose: 5-Hydroxytryptamine (5-HT) can enhance human ureteral contractions. However, the mediating receptors have not been clarified yet. The study sought to further characterize the mediating receptors using several more selective antagonists and agonists. Experimental approach: Human distal ureters were obtained from 88 patients undergoing cystectomy. The mRNA expression levels of 5-HT receptors were examined using RT-qPCR experiments. The phasic contractions of ureter strips, either spontaneous or evoked with neurokinin, were recorded in an organ bath. Key results: Among the 13 5-HT receptors, 5-HT2A and 5-HT2C had the highest mRNA expression levels. 5-HT (10-7–10-4 M) concentration-dependently increased the frequency and baseline tension of phasic contractions. However, a tachyphylaxis effect was observed. SB242084 (100 nM) and ketanserin (100 nM), which are 5-HT2C selective and non-selective antagonist, respectively, shifted the 5-HT concentration-response curves (frequency and baseline tension) rightward. 5-HT2C selective agonist, vabicaserin, increased contraction frequency with an Emax of 35% of 5-HT. 5-HT2A selective antagonist, volinanserin (100 nM), only reduced baseline tension. The selective antagonists of 5-HT1A,1B, 1D, 2B, 3, 4, 5, 6, and 7 had no antagonism. Blockade of voltage-gated sodium channels, α1-adrenergic receptors, adrenergic neurotransmission, and neurokinin-2 receptors using tetrodotoxin, tamsulosin, guanethidine, and Men10376, respectively, and desensitizing sensory afferents using capsaicin (100 μM), significantly reduced 5-HT effects. Conclusion and implications: 5-HT enhanced ureteral phasic contractions mainly by activating 5-HT2C. Activation of sympathetic nerve and sensory afferents partly contributed to 5-HT effects. 5-HT and 5-HT2C receptors could be promising targets for ureteral stone expulsion and ureteral colic relief.

The enhancing effect of 5-HT on phasic contractions of human isolated distal ureter and possible mediating mechanisms

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**Conflict of interest disclosure**

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

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Abstract**

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**Experimental approach:** Human distal ureters were obtained from 88 patients undergoing cystectomy. The mRNA expression levels of 5-HT receptors were examined using RT-qPCR experiments. The phasic contractions of ureter strips, either spontaneous or evoked with neurokinin, were recorded in an organ bath.

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antagonism. Blockade of voltage-gated sodium channels, α1-adrenergic receptors, adrenergic neurotransmission, and neurokinin-2 receptors using tetrodotoxin, tamsulosin, guanethidine, and Men10376, respectively, and desensitizing sensory afferents using capsaicin (100 μM), significantly reduced 5-HT effects.

**Conclusion and implications:** 5-HT enhanced ureteral phasic contractions mainly by activating 5-$HT_{2C}$. Activation of sympathetic nerve and sensory afferents partly contributed to 5-HT effects. 5-HT and 5-$HT_{2C}$ receptors could be promising targets for ureteral stone expulsion and ureteral colic relief.

**Key words** : 5-hydroxytryptamine, phasic contraction, 5-HT agonists and antagonists, 5-$HT_{2C}$ receptors, human distal ureter

**Bullet point summary**

*What is already known?* 5-HT increased human ureteral contractions. However, the mediating receptors have not been clarified.

*What does this study add?* (1) 5-$HT_{2C}$ receptors are the main mediating receptors for the enhancing effect of 5-HT on human ureter. (2) Activation of sympathetic nerves and sensory afferents partly contribute to 5-HT effects.

*What is the clinical significance?* 5-$HT_{2C}$receptors could be a promising target for ureteral stone expulsion and ureteral colic relief.

## Introduction

Ureteral contractions are mainly regulated by neurotransmitters released by sympathetic, parasympathetic nerves, and sensory afferents (Canda, Turna, Cinar & Nazli, 2007; Lim, Sellers & Chess-Williams, 2022). Although the adrenergic system (noradrenaline and its α1 receptor) is widely accepted to play a dominant role in ureteral contraction control, other mediators, such as 5-hydroxytryptamine (5-HT), have been shown to exert marked effects in ureteral contractions (Canda, Turna, Cinar & Nazli, 2007). 5-HT has similar potency as that of α1-receptor agonist, phenylephrine, and much greater potency than muscarinic receptor agonist, acetylcholine, in stimulating porcine ureteral contractions (Lim, Chess-Williams & Sellers, 2018a; Lim, Chess-Williams & Sellers, 2020).

The vital role of 5-HT in the regulation of ureteral contraction has been revealed in ureteral preparations from different species. Enhanced ureteral contractions induced by 5-HT presented as increase in the frequency or elevated tension of phasic contractions have been observed in ureters from pigs (Hauser et al., 2002; Hernandez et al., 2003; Lim, Chess-Williams & Sellers, 2018b; Lim, Chess-Williams & Sellers, 2020) and humans (Gidener, Gumustekin & Kirkali, 1999; Gidener, Kirkali & Guven, 1995; Kuwahara, 1983; Long & Nergardh, 1978). However, no effects of 5-HT have been observed in canine and rabbit ureters (Borgstedt, Emmel & Benjamin, 1966; Yalcin et al., 2013), and no effects or dose-dependent effects (high doses inhibit and low doses excite) have been reported in rat ureters (Ancill, Jackson & Redfern, 1972). Clearly, a species difference exists in 5-HT actions in ureteral contractions. Ideally, human ureters should be the best for the study of 5-HT physiology and pharmacology, because the final purpose for the study of ureteral contraction is to help identify potential therapeutic agents for kidney stone expulsion or ureteral colic relief in humans (Canda, Turna, Cinar & Nazli, 2007; Lim, Sellers & Chess-Williams, 2022). However, to our knowledge, few studies have reported the role of 5-HT in human ureteral contractions(Gidener, Gumustekin & Kirkali, 1999; Gidener, Kirkali & Guven, 1995; Long & Nergardh, 1978; Roedel, Ravens, Kasper, Wirth, Jepps & Propping, 2018).

5-HT receptors are classified into seven subtypes (5-$HT_1$to 5-$HT_7$) (Barnes et al., 2021; Gothert, 2013; Gothert, Bonisch, Malinowska & Schlicker, 2020), which include G-protein-coupled receptors and ion channels. 5-$HT_1$($1_A$,$1_B$,$1_D$,$1_E$, $1_F$) and 5-$HT_5$ subtypes are coupled to Gi/o and inhibit the cyclic adenosine 3,5-monophosphate (cAMP). Whereas 5-$HT_4$, 5-$HT_6$, and 5-$HT_7$are Gs-coupled receptors that increase cAMP activity. 5-$HT_2$ subtypes (2A, 2B, and 2C) are coupled to Gq; they activate phospholipase C to increase intracellular $Ca^{2+}$. 5-$HT_3$ subtypes are the ligand-gated cation channels.

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5-$HT_2$ receptors, particularly the 5-$HT_{2A}$subtype, which are located on smooth muscles, have been shown to be the predominant receptor for 5-HT-induced ureteral contractions in porcine ureters (Hauser et al., 2002; Hernandez et al., 2003; Lim, Chess-Williams & Sellers, 2018b). Intravenous and topical application of the 5-$HT_{2A}$ agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), increased the frequency of pig ureteral contractions *in vivo* , which were reduced by the 5-$HT_2$ receptor antagonist, ketanserin or methysergide (Hauser et al., 2002). 5-HT-induced increase in the tension of isolated porcine intravesical ureters was reduced by 5-$HT_2$ and 5-$HT_{2A}$ receptor antagonists, ritanserine and spiperone (Hernandez et al., 2003). The 5-$HT_2$ agonist, α-methyl-5HT, increased phasic contractions and overall contractile activity in isolated porcine distal ureter, which could be inhibited by the 5-$HT_2$ receptor antagonist, ketanserin (Lim, Chess-Williams & Sellers, 2018b). However, in human ureters, the role of 5-$HT_2$ has not been confirmed.

Long & Nergardh (1978) (Long & Nergardh, 1978) demonstrated that 5-HT evoked a concentration-dependent increase in contractions in isolated human ureter strips, which could be blocked by methysergide, a mixed 5-$HT_1/2_A/2_C$ receptor antagonist. In line with this finding, Gidener et al (1995). reported an inhibition of 5-HT-evoked human ureteral contractions by methysergide and ketanserin (Gidener, Kirkali & Guven, 1995). However, in a later study, Gidener et al (1999). failed to reveal the involvement of 5-$HT_2$ receptors (Gidener, Gumustekin & Kirkali, 1999). They found that 5-$HT_2$receptor agonist, DOI, could not evoke contractile effects and ketanserine (100 nM) had a weak antagonistic effect on 5-HT-induced ureteral contractions (Gidener, Gumustekin & Kirkali, 1999). Therefore, the exact receptors for 5-HT-induced contractile response in human ureter could not be clearly defined.

The uncertainty may due to the low potency and selectivity of antagonists and agonists used in previous studies. For example, the DOI could not discriminate the three subtypes of $5HT_2$, and the most frequently used 5-$HT_2$ antagonist, ketanserin, could block 5-$HT_{2A}$ and 5-$HT_{2C}$ (Barnes et al., 2021; Hernandez et al., 2003). The most reliable method known for functional determination of the 5-HT receptor subtype mediating a given effect is dependent on the availability of selective antagonists of multiple 5-HT receptor subtypes (Barnes et al., 2021; Matsumoto-Miyai, Yoshizumi & Kawatani, 2015). Over 20 years passed since after Gidener et al characterizing the mediating 5-HT receptor subtypes in human ureter (Gidener, Gumustekin & Kirkali, 1999; Gidener, Kirkali & Guven, 1995), and many highly selective agonists and antagonists for $5HT_2$ and other subtypes have been discovered (Barnes et al., 2021). Therefore, this study mainly sought to further examine 5-HT effects and characterize the subtypes mediating 5-HT activity in human ureters using highly selective agonists and antagonists discovered in recent years.

A strong tachyphylaxis of the human ureter to 5-HT was found in our preliminary experiments and in one previous study (Hernandez et al., 2003). However, the effects of 5-HT and various antagonists in previous human ureter studies were based on the cumulative concentration-response curves (Gidener, Gumustekin & Kirkali, 1999; Gidener, Kirkali & Guven, 1995) ,which may lead to inaccurate analysis due to the tachyphylaxis. Therefore, another aim of our study was to re-examine the effects of 5-HT and various antagonists with the no-cumulative concentration-response curves of 5-HT.

Since none of the 5-HT receptor antagonists has significant blocking effect on 5-HT-induced contractions in human ureters (Gidener, Gumustekin & Kirkali, 1999; Gidener, Kirkali & Guven, 1995) , studies on porcine ureters (Hernandez et al., 2003) indicated that parts of the 5-HT-induced contractions were indirectly mediated by noradrenaline (NA) release from sympathetic nerves (Hernandez et al., 2003). Therefore, the involvement of sympathetic and sensory neurotransmission, the two most important regulatory mechanisms for ureter contractions, in 5-HT-induced effects were examined in this study.

## Materials and Methods

### 2.1 Ureter strip preparation

All experimental procedures were approved by the Ethics Committee of the Second Hospital of Shandong University (KYLL-2022LW120). All patients provided written informed ethical consents before participating in the study. Distal ureters were obtained from 88 patients (52 men and 36 women; mean age = 56±12.1 years; range = 49–71 years) undergoing cystectomy for bladder cancer. Surgeries were performed in the Department

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of Urology, The Second Hospital of Shandong University and the Department of Urology, Shandong Provincial Hospital between October 2020 and June 2022. All tissue specimens appeared macroscopically normal with no sign of tumor, obstruction, inflammation, or any other changes. The distal ureter, approximately 5 cm long, was excised 2 cm away from the orifices in the urinary bladder. Ureteral tissues were immediately transported to the laboratory, where the surrounding vascular, adipose, and connective tissues were carefully removed. Longitudinal segments (10 mm long) were isolated from each ureter for organ bath experiments. Small portions of the tissues were used for PCR experiments.

Under isoflurane anesthesia, the distal ureters from adult Sprague Dawley rats (200–300g), C57BL/6 mice (30–40 g), and Hartley guinea pigs (300–400 g) were isolated. Female animals were selected, since no studies indicated sex difference in ureteral contractions. All the animals were obtained from Wugyue Animal Company (Jinan, China). All animals were kept in individually ventilated cages in a 12/12h light/dark cycle with controlled temperature and humidity and food and water ad libitum. All animal experimental procedures were approved by the Ethics Committee of the Second Hospital of Shandong University (KYLL-2022A130). Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology.*

### 2.2 Organ bath experiments

Ureteral specimens were placed in warm Krebs solution, which was composed of NaCl, 118 mM; KCl, 4.7 mM, $CaCl_2$, 1.9 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 24.9 mM; $KH_2PO_4$, 1.2 mM, and glucose, 11.7 mM at a pH of 7.4. Longitudinal ureteral strips (length, 10±1.5 mm; width, 2–3 mm) were tied at each end using a fine thread and mounted in a vertical organ bath in Krebs solution (volume, 10 ml). Thereafter, the Krebs solution was heated to 37 in a circulating warm water bath and continuously gassed with 95% $O_2$ and 5% $CO_2$. The longitudinal tension of the strips was continuously recorded with an isometric transducer and processed using Lab Chart 7 software (AD Instruments Pty Ltd., New South Wales, Australia). Tissues were pre-stretched to a baseline tension of 10 mN (1 g) for human ureter and 0.1mN for rat, mouse, and guinea pig ureters. The contractions were allowed to equilibrate for 20–30 min until stable spontaneous contractions were observed. In strips with no spontaneous contractions, neurokinin A (NKA, 10–30 nM) was applied to initiate contractions. Strips that had spontaneous or NKA-evoked contractions were considered as having good viability; otherwise, they were discarded.

Due to the strong tachyphylaxis of ureteral tissue to 5-HT (Fig. 1), the concentration-response curve could not be constructed for the same strips. Therefore, only one concentration of 5-HT was applied to each ureteral strip, particularly for higher concentrations (>1 μM). A concentration–response curve was constructed first on strips from the same ureteral tissue. Thereafter, the final 5-HT concentration-response curve was constructed based on the average of five to six ureters. The antagonism study was conducted in two preparations from the same ureter tissue in parallel; one preparation was treated with 5-HT, and the other one was treated with the antagonist for 15 min before 5-HT was added.

### 2.3 RT-qPCR

The specimens were frozen in liquid nitrogen and stored at –80 before the experiment. Total RNA was extracted using the RNA Simple Total RNA kit (Tiangen, Beijing, China). The RNA concentration was determined using an ultraviolet spectrophotometer. Reverse transcription was performed using a SPARKscript II RT plus Mix kit (Sparkjade, Qingdao, China), according to the manufacturer's instructions, and complimentary-DNA was amplified (40 cycles of denaturation for 15 s at 95°C, and primer annealing and elongation for 30 s at 60°C). RT-qPCR was performed using a SYBR Green qPCR Mix (Sparkjade) and an QuantStudio 5 system (Thermo Fisher, Waltham, MA, USA). Selective primers for 5-HT receptors were generated by BioSune (Shanghai, China), and the sequences of primers are shown in Table 1. Expression was measured using the $2^{-t}$ method.

### 2.4 Chemicals

The chemicals used in this study include 5-HT hydrochloride (MCE), p-MPPI (MCE, 5-$HT_{1A}$ selective antag-

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onist), SB-224289 hydrochloride (MCE, $HT_{1B}$ selective antagonist), LY310762(MCE, $HT_{1D}$selective antagonist), volinanserin (MCE, 5-$HT_{2A}$selective antagonist), RS-127445(MCE, 5-$HT_{2B}$ selective antagonist), SB-242084 (MCE, 5-$HT_{2C}$ selective antagonist), ketanserin (MCE, 5-$HT_2$ non-selective antagonist), ondansetron hydrochloride dehydrate (Sigma-Aldrich, 5-$HT_3$ selective antagonist), GR113808 (Tocris, 5-$HT_4$ selective antagonist), SB 699551 (Sigma-Aldrich, 5-$HT_5$ selective antagonist), SB-399885 hydrochloride (Sigma-Aldrich, 5-$HT_6$ selective antagonist), SB-269970 hydrochloride (MCE, 5-$HT_7$ selective antagonist), 25CN-NBOH hydrochloride (Efebio, 5-$HT_{2A}$ selective agonist), vabicaserin hydrochloride (MCE, 5-$HT_{2C}$selective agonist), tamsulosin (MCE, $\alpha_1$-adrenergic receptor antagonist), guanethidine sulfate (MCE, adrenergic neurotransmission blocker), Men10376 (MCE, NK-2 receptor antagonist), neurokinin A (NKA, MCE), and tetrodotoxin (TTX, Sigma-Aldrich). Stock solutions of 5-HT, TTX, Men10376, and guanethidine sulfate were prepared in distilled water, and the stock solutions of the other chemicals were prepared in dimethyl sulfoxide (DMSO). DMSO (0.05% final concentration) did not significantly change the activity of ureteral strips. Based on the affinity values published in the literature, concentrations that would produce significant blockade of the selective receptor subtype (near saturation) were chosen, without an action at the other receptor subtypes.

### 2.5 Data analysis

The data and statistical analysis comply with the recommendations of the*British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2022). All data are presented as mean±S.E.M and analyzed using the Sigmaplot 14.0 software (California, USA). Because our preliminary data showed that 5-HT mainly increases the frequency and baseline tension of the phasic contractions but does increase the amplitude of each contraction, the effects of 5-HT were measured as percentage change in the frequency or baseline tension from the control. Frequency or baseline tension was measured in 3-min intervals immediately before and after 5-HT application. 5-HT concentration-response curves were constructed based on the average data from 5–6 ureters. The concentration required to produce half-maximal response ($EC_{50}$) for 5-HT or related agonists was estimated using the Hill equation: % response = (MAX response / [drug concentration + $EC_{50}$]) n, where MAX response ($E_{max}$) = maximal % response, drug concentration = concentration of the agonists, $EC_{50}$ = the half-maximal concentration, and n = the Hill coefficient. $pD_2$ was defined as the negative logarithm of $EC_{50}$ ($pD_2$ = –log$EC_{50}$[M]). Statistical significance was tested using student t-test or paired two-tailed t-test, with a layered Bonferroni post-hoc test for multiple comparisons, when appropriate. Data were considered statistically significant, when $p < 0.05$.

### 2.6 Nomenclature of targets and ligands

The nomenclature of 5-HT receptors and key protein ligands comply with the recommendations made *by British Journal of Pharmacology* and are archived in the Concise Guide to PHARMACOLOGY (Alexander et al., 2021).

## Results

The spontaneous contractions were observed in 22% (19 of 88) of the isolated distal ureters. In strips with no spontaneous contractions, neurokinin A (NKA, 10–30 nM) was administered to initiate contractions. Our preliminary experiments showed that both spontaneous and NKA-evoked contractions were enhanced by 5-HT, which were demonstrated by an increase in frequency and rise in baseline tension. To simultaneously observe the effects of 5-HT on both the frequency and baseline tension, all the experiments were conducted on ureter strips with spontaneous or NKA-induced (10–30 nM) phasic contractions.

### 3.1 Desensitization effect of 5-HT

In our preliminary experiments, significant tachyphylaxis effects of 5-HT were observed in human ureters. To further examine the tachyphylaxis effects, 10 μM of 5-HT was used as a test contraction. First time application of 10 μM 5-HT evoked a marked increase in frequency (81±5% increase) and baseline tension (54±3% increase, n=6 strips) (Fig. 1Aa). After 30–40 min washout, second time application of 10 μM 5-HT evoked a much lower response (8±2% increase in frequency, 9±2% increase in baseline tension, Fig. 1Aa and 1B). Furthermore, 10 μM 5-HT evoked response was significantly reduced when it was applied after 1 μM

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5-HT (Fig. 1Ab and 1B).

### 3.2 Concentration-response of 5-HT

Due to the strong tachyphylaxis, only one concentration of 5-HT was applied to each strip particularly at concentrations >1 μM to construct the concentration-response curve. 5-HT at concentrations of 100 nM–100 μM induced increases in the frequency and basal tension of the phasic contractions [Fig. 2A]. For frequency increase, analysis of the dose-response curve revealed an $EC_{50}$ of 2.96 μM [Fig. 2B]. For baseline tension increase, analysis of the dose-response curve revealed an $EC_{50}$ of 10.92 μM [Fig. 2B].

### 3.3 mRNA expression of 5-HT receptors in human ureter

The mRNA expression levels of 5-HT receptors in human ureter were examined using RT-qPCR experiments. Among all 13 5-HT receptors ($1_{A,B,D,E,F}$, $2_{A,B,C}$, 3, 4, 5, 6, 7), the relative expression levels of 5-$HT_{2A}$, 5-$HT_{2C}$, and 5-$HT_{1A}$ were the highest in intact ureter (Fig. 3). Those of 5-$HT_{2B}$, 5-$HT_{1F}$, 5-$HT_{1E}$, 5-$HT_{1B}$ and 5-$HT_7$ were moderate, whereas those of 5-$HT_{3,4,5}$ and 5-$HT_6$ were the lowest.

### 3.4 Effects of 5-HT receptor antagonists

Previous studies in porcine ureters indicated that 5-$HT_2$was the main mediating receptor for 5-HT stimulatory actions. mRNA expression of 2A and 2C receptors were higher in the PCR experiments [Fig. 3]. Therefore, the contribution of these two 5-HT subtype receptors with their selective antagonists were examined. In the presence of 5-$HT_{2A}$selective antagonist (volinanserin) and 5-$HT_{2C}$ selective antagonist (SB242084) at their saturating concentration (100 nM), 5-HT concentration-response curve for baseline tension was shifted rightward (Fig. 4A and 4B). However, only 5-$HT_{2C}$ antagonist, SB242084, rightward shifted 5-HT concentration-response curve for frequency (Fig 4B, Table 2), and 5-$HT_{2A}$antagonist, volinanserin, had no significant effect on 5-HT-induced frequency change [Fig. 4A, Table 2].

Ketanserin is a 5-HT receptor non-selective antagonist that can block both 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors (Barnes et al., 2021) (Hauser et al., 2002). Consistent with the findings from porcine ureter studies (Hauser et al., 2002; Hernandez et al., 2003; Lim, Chess-Williams & Sellers, 2018b), ketanserin (100 nM) shifted 5-HT concentration-response curves rightward both for baseline tension and frequency [Fig. 4C]. A significant change in $EC_{50}$ and $E_{max}$occurred for both frequency and tension changes in presence and absence of ketanserin ($p < 0.05$, Table 2).

Other receptor subtype selective antagonists, p-MPPI (100 nM, 5-$HT_{1A}$ selective), SB-224289 (100 nM, 5-$HT_{1B}$ selective), LY310762 (3 μM, 5-$HT_{1D}$ selective), RS-127445 (100 nM, 5-$HT_{2B}$selective), ondansetron (30 nM, 5-$HT_3$ selective), GR-113808 (100 nM, 5-$HT_4$ selective), SB 699551 (10 nM, 5-$HT_5$ selective), SB 399885 (100 nM, 5-$HT_6$ selective), and SB 269970 (10 nM, 5-$HT_7$ selective), have no antagonistic effects on 5-HT-induced contractions. Mean $EC_{50}$ values and maximum responses for frequency or tension changes were similar in the absence and presence of each antagonist ($p > 0.05$, Table 2).

### 3.5 Effects of5-$HT_{2A}$and 5-$HT_{2C}$ receptor agonists

These results suggest that 5-$HT_{2A}$ and 5-$HT_{2c}$ may be the predominant receptors for 5-HT stimulatory action. We further tested the effects of their agonists. 5-$HT_{2C}$-selective agonist, vabicaserin, dependently increased the frequency of phasic contractions [Fig. 5]. However, the enhancing effect was much less than that of 5-HT, and the $E_{max}$ was 35% of 5-HT. The effect of 5-$HT_{2A}$-selective agonist, 25CN-NBOH, was much less than that of 5-$HT_{2C}$ agonists in increasing the contraction frequency (Fig. 5). Moreover, both 5-$HT_{2A}$ and 5-$HT_{2c}$ agonists did not increase the baseline tension.

### 3.6 Εφφεςτς οφ α-ρεςεπτορ ανδ NK-2 ρεςεπτορ ανταγονιστ

These observations indicate that no one 5-HT receptor antagonist could completely block 5-HT-induced responses. A previous study on porcine ureter suggested the involvement of noradrenaline (NA) release from sympathetic nerves (Hernandez et al., 2003). Thereafter, we examined the effect of α-adrenergic receptor antagonist, tamsulosin, and adrenergic neurotransmission blocker, guanethidine, on 5-HT stimulatory effect.

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In the presence of tamsulosin (10 μM) and guanethidine (10 μM), 5-HT-induced enhancement in frequency and baseline tension was significantly reduced. (Fig. 6 and Table 2). Additionally, consistent with previous study in pig ureter, this study found that tetrodotoxin (TTX, 1 μM) significantly attenuated 5-HT effects (Fig. 6C and Table 2), suggesting the activation of intramural nerves.

A study on human ureter reported that neurokinin released from sensory afferents increased ureteral contractions by activating NK-2 receptors (Patacchini, Santicioli, Zagorodnyuk, Lazzeri, Turini & Maggi, 1998). To examine the involvement of sensory nerves and the releasing neurokinin, the effect of NK-2 receptor antagonist, MEN10376, was examined in ureter strips with spontaneous contractions. MEN10376 (1 μM) shifted 5-HT concentration-response curves (both frequency and baseline tension) rightward (Fig. 7A). Desensitization of sensory afferents with capsaicin (100 μM) led 5-HT to completely lose its stimulatory effect in increasing frequency (Fig. 7B top figure) and significantly reduced 5-HT-induced baseline tension increase (Fig. 7B low figure).

### 3.7 Species difference

Rat, mouse, and guinea pig are commonly used animals in preclinical experiments. In this study, 5-HT effects on distal ureteral contractions from these animals were compared with those of human ureter under the same recording conditions. 5-HT (10 μM) induced an enhancement effect on human ureter. However, 5-HT (10 μM) decreased rat ureteral contractions and had no effect on guinea pig and mouse ureteral contractions (Fig. 8).

## Discussion and Conclusions

In this study, the enhancing effect of 5-HT on phasic contractions of human distal ureter and potential underlying mechanisms were examined. These were our main finding: (1) 5-HT concentration dependently increased the phasic contractions of human ureter both in frequency and baseline tension. (2) Among the 13 5-HT receptors, the mRNA expression levels of 5-$HT_{2A}$and 5-$HT_{2C}$ were the highest. (3) Only the 5-$HT_{2C}$or 5-$HT_{2A}$-selective antagonists had blocking effects on 5-HT stimulatory action. (4) 5-$HT_{2C}$ but not 5-$HT_{2A}$ selective agonist could increase the frequency of contraction in a concentration-dependent manner, but with less potency than 5-HT. (5) Blocking adrenergic and sensory neurotransmission significantly attenuated 5-HT stimulatory effects. These results suggest that 5-HT enhances human ureteral phasic contractions mainly by activating 5-$HT_{2C}$. Activation of sympathetic nerves and sensory nerves partly mediates 5-HT effects by noradrenaline and neurokinin release.

In most of the studies on ureters of various species, including humans, the pharmacology of 5-HT was usually examined using cumulative concentration-response curves (Gidener, Gumustekin & Kirkali, 1999; Gidener, Kirkali & Guven, 1995). In our study, a significant desensitization effect of 5-HT was revealed (Fig. 2). Therefore, to establish the 5-HT concentration-response curves, only one concentration of 5-HT was applied to each ureter strip, particularly for high concentrations (>1 μM). The $EC_{50}$ value of 5-HT (2–10 μM) obtained in this study (Fig. 2) was one order less than that of a previous report (around 20 μM) in human ureter (Gidener, Kirkali & Guven, 1995), suggesting the influence of tachyphylaxis on previous analysis. When the blocking effects of the antagonists were investigated, two preparations from the same ureter tissue were examined in parallel to prevent the desensitization effect. One was treated with 5-HT, and the other was treated with the antagonist before 5-HT application. To our knowledge, this is the first study to examine the desensitization effects of 5-HT on ureter in detail.

Our results generally corroborate findings from previous studies on human and porcine ureters suggesting that 5-$HT_2$ mediates 5-HT-induced responses. However, several lines of evidence indicated that 5-$HT_{2C}$ are the main subtype in mediating 5-HT responses. Firstly, 5-$HT_{2C}$mRNA expression levels are highly expressed in human ureter (Fig. 2). Secondly, 5-$HT_{2C}$-selective antagonist has significant blocking effect on 5-HT-induced increase in frequency and baseline tension, and ketanserin, an unselective antagonist of 5-$HT_{2C}$, has a blocking effect (Fig. 4). Thirdly, 5-$HT_{2C}$-selective agonists have similar effects as 5-HT in increasing the frequency of ureteral contractions, although the potency is only 35% of 5-HT. Finally, other 5-HT receptor (5-$HT_{1,\ 2A,\ 2B,\ 3,\ 4,\ 5,\ 6}$ and $_7$) selective antagonists has weak or no blocking effects.

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Our observations that 5-$HT_{2A}$-selective antagonists have weaker blocking effect than 5-$HT_{2C}$-selective antagonists and 5-$HT_{2A}$-selective agonists have no stimulatory effect suggest that 5-$HT_{2A}$ plays a minimal role than 5-$HT_{2C}$ in mediating 5-HT-induced effects. Our conclusion contrasts with those of porcine ureter studies showing that 5-$HT_{2A}$ is the main mediatory receptor (Hernandez et al., 2003; Lim, Chess-Williams & Sellers, 2018b; Lim, Chess-Williams & Sellers, 2020). This inconsistency between human and pig ureters may suggest a species difference. Probably, the non-selective antagonist or agonist of 5-$HT_{2A}$ (katanserin and DOI) has been used in previous porcine studies (Hauser et al., 2002; Lim, Chess-Williams & Sellers, 2020). Whereas, katanserin or DOI could bind both 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors (Barnes et al., 2021; Hernandez et al., 2003). In our study, katanserin had similar blocking effect as that of the selective antagonist of 5-$HT_{2C}$, which may indicate that the blocking effect of katanserin may be mainly mediated by its antagonism of 5-$HT_{2C}$ receptors.

For 5-$HT_4$, 5-$HT_6$, and 5-$HT_7$ receptor subtypes, since these receptors are known to mediate relaxation in smooth muscles by adenylyl cyclase inhibition and have lower levels of mRNA expression in human ureter (Fig. 2), expectedly, no blocking effects of these receptor selective antagonists (5-$HT_{4,\,6,\,7}$) were demonstrated on 5-HT's stimulatory effect.

In terms of the 5-$HT_1$ ($_{1A,1B,1D}$) and 5-$HT_5$ subtypes, they could be involved in 5-HT-induced contractions because they are coupled to Gi/o and inhibit cAMP formation. However, the lower expression of 5-$HT_5$ and absent blocking effect of its selective antagonist excluded 5-$HT_5$ involvement. Although 5-$HT_1$($_{1A,1B,1D}$) expression is relatively high (Fig. 2), 5-$HT_1$ non-selective agonist, 5-CT, has been reported to evoke ureteral contraction in human ureter (Gidener, Kirkali & Guven, 1995). However, no blocking effects were observed with the selective antagonist of 5-$HT_{1A,1B,1D}$ in our study (Table 2), indicating that these receptors do not seem to be involved in 5-HT responses.

5-$HT_3$ subtypes are the only 5-HT ligand-gated cation channels. 5-$HT_3$ receptor-selective antagonist, ondansetron, was found to have acceptable pain-relieving properties in patients with acute ureteral colic, suggesting that 5-$HT_3$ receptors may play an important role in human ureteral function (Ergene, Pekdemir, Canda, Kirkali, Fowler & Coskun, 2001). However, the low expression of 5-$HT_3$ receptors in human ureter and no blocking effect of ondansetron exclude 5-$HT_3$ receptor involvement in human ureter.

Consistent with a previous study on porcine ureter (Hernandez et al., 2003), our study found that the 5-HT stimulatory effect on human ureter was significantly reduced in the presence of α-adrenergic receptor antagonist, tamsulosin, and adrenergic neurotransmission blocker, guanethidine (Fig. 6). This suggest that part of the 5-HT stimulatory effect is mediated by NA release from adrenergic nerves acting on α-adrenoceptors. This is further supported by the observation that blocking intramural nerve activation with tetrodotoxin significantly attenuated 5-HT stimulatory effects [Fig. 6]. 5-$HT_{2C}$or 5-$HT_{2A}$ could act as prejunctional receptors in autonomic excitatory nerve terminals to facilitate the neurogenic contraction of the detrusor (Matsumoto-Miyai, Yoshizumi & Kawatani, 2015). NA could increase ureteral contractions through α1 and α2 adrenoceptors (Hertle & Nawrath, 1984). Therefore, NA release in response to 5-HT stimulation could contribute to the contractile effect of 5-HT. However, the exact mechanisms are not clear. Putative prejunctional excitatory 5-HT receptors, such as 5-$HT_1$, 5-$HT_3$, and 5-$HT_4$, seem not cause NA release, because their selective antagonists have no blocking effects on 5-HT effects. The indirect effects of 5-HT in inducing NA release have been proposed in porcine ureter (Hernandez et al., 2003). This indirect mechanism may be present in human ureter.

According to our study, 5-HT stimulatory responses were significantly attenuated by NK-2 receptor antagonist, MEN10376, by pretreatment with large concentration of capsaicin to desensitize sensory afferents and by TTX treatment. These findings suggest the involvement of sensory afferent activation in response to 5-HT stimulation. This is the first time to reveal sensory afferent involvement in 5-HT-induced responses in human ureter. The important role of sensory afferents and tachykinin release by stimulating NK2 receptors have been demonstrated in human ureter (Patacchini, Santicioli, Zagorodnyuk, Lazzeri, Turini & Maggi, 1998). 5-HT could produce a concentration-dependent depolarization and an increase in action potential firing in dorsal root ganglion (DRG) neurons via 5-$HT_{2A}$ and 5-HT $_{2C}$ receptors (Salzer, Gantumur, Yousuf &

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Boehm, 2016; Todorovic & Anderson, 1990). Therefore, 5-HT stimulation could result in tachykinin release in sensory afferent nerves and could contribute to the stimulatory effect of 5-HT by acting on NK2 receptors present in ureteral smooth muscles.

Under the same recording conditions as those of human ureter, guinea pig and mouse ureter had no response to 5-HT (10 μM) (Fig. 8). Other studies reported no effect of 5-HT in isolated rabbit ureter (Yalcin et al., 2013). Notably, a significant suppressive effect of 5-HT was found in the rat ureter (Fig. 8). Clearly, 5-HT response in ureter differs among different species. Given the similar response patterns of 5-HT between pig and human ureter, pig ureter has been suggested as a good model for human ureter (Lim, Chess-Williams & Sellers, 2020). In human ureter, 5-HT induced similar enhancement effects, and they are mainly mediated by 5-HT2 receptors, as in porcine ureters (Hauser et al., 2002; Hernandez et al., 2003; Lim, Chess-Williams & Sellers, 2018b; Lim, Chess-Williams & Sellers, 2020). Therefore, porcine ureters should be better than rat, mouse, guinea pig, and rabbit ureters for preclinical study of 5-HT pharmacology. Notably, the 5-HT effects and mediating receptors between pig and human ureter differed. In porcine distal ureter, 5-HT stimulation did not induce baseline tone increase. $5\text{-HT}_{2A}$ has been suggested as the main mediating receptor (Lim, Chess-Williams & Sellers, 2018a), which is different from human ureter.

In conclusion, our results corroborate previous human and porcine ureter study findings that suggest that $5\text{-HT}_2$ contributes to 5-HT-induced responses. Our results further suggest that $5\text{-HT}_{2C}$ plays a more important role than $5\text{-HT}_{2A}$ in mediating 5-HT enhancing effects on human ureter. Additionally, activation of sympathetic nerve or activation of primary sensory afferents partly contributed. Given the prominent stimulatory effect of 5-HT on human distal ureter, and distal ureter as the most common location for lodgment of kidney stone (El-Barky, Ali, Sahsah, Terra & Kehinde, 2014), targeting 5-HT or $5\text{-HT}_{2C}$receptors could be a promising strategy for the management of ureteral stone expulsion and ureteral colic relief.

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**Conflict of interest**

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

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**Figure legends**

**Fig. 1. The tachyphylaxis effects of 5-HT. (A)** Typical recordings of human ureteral phasic contractions showing first time application of 10 μM 5-HT evoked a marked increase in frequency and baseline tension. However, second time application of 10 μM 5-HT evoked a much smaller response, even after 40 min washout (Aa). Furthermore, 10 μM 5-HT-evoked response was significantly reduced, when it was applied after 1 μM 5-HT (Ab). (**B** ) The summary data (n=5 ureter strips) of 10 μM 5-HT induced a percentage increase in frequency and baseline tension either applied alone or after 1 or 10 μM 5-HT. Frequency or baseline tension was measured in 3-min intervals immediately before and after 5-HT application.

**Fig. 2. 5-HT concentration dependently increased the phasic contractions of human distal ureter. (A)** Typical recordings showing 5-HT concentration (100 nM–100 μM) dependently increased the phasic contractions both in frequency and baseline tension. (**B** ) 5-HT concentration-response curves for frequency and baseline tension. Contractions are expressed as a percentage of the calculated maximal effect. The curves were constructed from recordings of six ureter strips and fitted with Hill equation to reveal an $EC_{50}$ of 2.9 μM and 10.9 μM for frequency and baseline, respectively.

**Fig. 3. The relative mRNA expression level of 5-HT receptors in human distal ureter.** Total RNA was extracted from human distal ureter tissue, and RT-qPCR was performed. The primer sets for the 13 5-HT receptors were indicated in Table 1. Because the expression level of 5-HT receptor was much lower than that of beta-actin, the mRNA expression of each receptor was expressed as a relative level to that of the highest expression in all receptors. mRNA expression of 5-$HT_{2A}$ and 5-$HT_{2C}$ are the highest, and those of 5-$HT_{1A}$, $_{2B,\ 1F,\ 1E,\ 1B,}$ and$_7$ are moderate. Summary data are the average from six experiments.

**Fig. 4. The blocking effect of 5-$HT_{2A}$ and 5-$HT_{2C}$ selective antagonists on 5-HT stimulatory action.** 5-HT concentration-response curves were established in the presence or absence of volinanserin (100 nM, 5-$HT_{2A}$ selective antagonist) (**A** ), SB 242084 (100 nM, 5-$HT_{2C}$ selective antagonist) (**B** ) and ketanserin (100 nM, 5-$HT_{2A}$and $_{2C}$ non-selective antagonist) (**C** ). SB 242084 and ketanserin shifted the 5-HT centration-response curve for both frequency and baseline tension rightward. However, volinanserin only shifted the 5-HT centration-response curve of baseline tension rightward. Contractions are expressed as a percentage of the calculated maximal effect. Each curve was the average data of five ureter strips.

**Fig. 5. 5-$HT_{2C}$ selective agonist concentration dependently increased the frequency of ureter phasic contractions.**Concentration-response curves to 5-HT, vabicaserin (5-$HT_{2C}$ selective agonist) and 25CN-NBOH (5-$HT_{2A}$selective agonist) were established. Both vabicaserin and 25CN-NBOH did not induce baseline increase; and only frequency changes were analyzed. The maximal response induced by vabicaserin and 25CN-NBOH is 35% and 4.8% of 5-HT, respectively. Each curve was the average data of five ureter strips.

**Fig. 6. Blocking sympathetic transmission significantly reduced 5-HT stimulating effect** . 5-HT concentration-response curves were established in the presence or absence of tamsulosin (10 μM, α1-adrenergic receptor antagonist) (**A** ), Guanethidine (10 μM, adrenergic transmission blocker) (**B** ) and TTX

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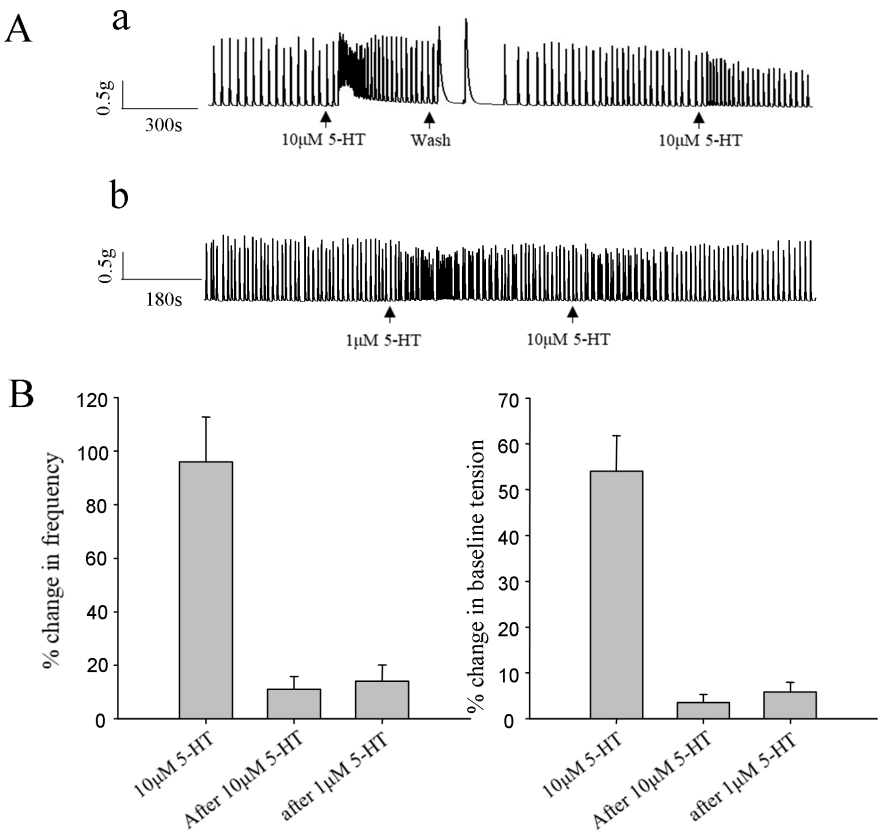
(1 μM, $Na^+$ channel blocker) **(C** ) Tamsulosin, guanethidine, and TTX all shifted 5-HT centration-response curve rightward for either frequency or baseline tension. Contractions are expressed as a percentage of the calculated maximal effect. Each curve was the average data of five ureter strips.

**Fig. 7. Blocking neurokinin receptor-2 (NK-2) or desensitizing sensory afferents with capsaicin significantly reduced 5-HT stimulatory effect** . 5-HT concentration-response curves were established in the presence or absence of Men10376 (1 μM, a NK-2 receptor antagonist)**(A)** or after desensitizing sensory afferents with capsaicin (100 μM) (**B** ). Men10376 shifted 5-HT centration-response curve rightward for either frequency or baseline tension. Pretreatment with capsaicin led 5-HT to completely lose the effect in increasing frequency (B, top), and shift 5-HT concentration-response curve of baseline tension rightward (B, low). Each curve was the average data of five ureter strips. KCl (80 mM) was applied to prove that the strips still have a good viability after capsaicin

**Φιγ. 8. Τηε διφφερεντ ρεσπονσες εοκεδ βψ 10μM 5-HT ιν ηυμαν, ρατ, γυινεα πιγ ανδ μουσε δισταλ υρετερ.** Ureteral contraction recordings were performed under the same conditions for all species. 5-HT (10 μM) induced an enhancement in human ureter but decreased rat ureteral contraction. It had no effect on guinea pig and mouse ureter.



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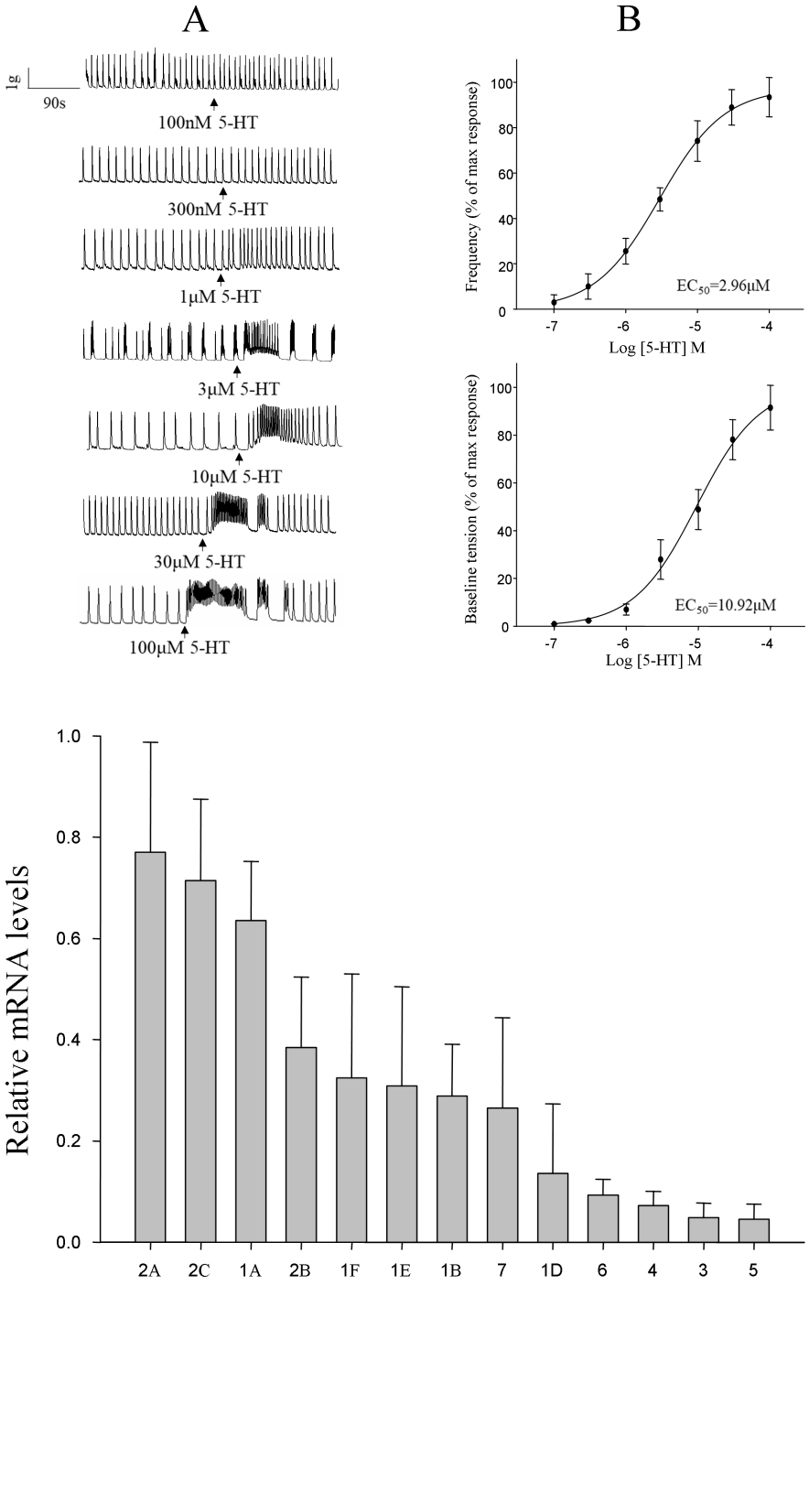

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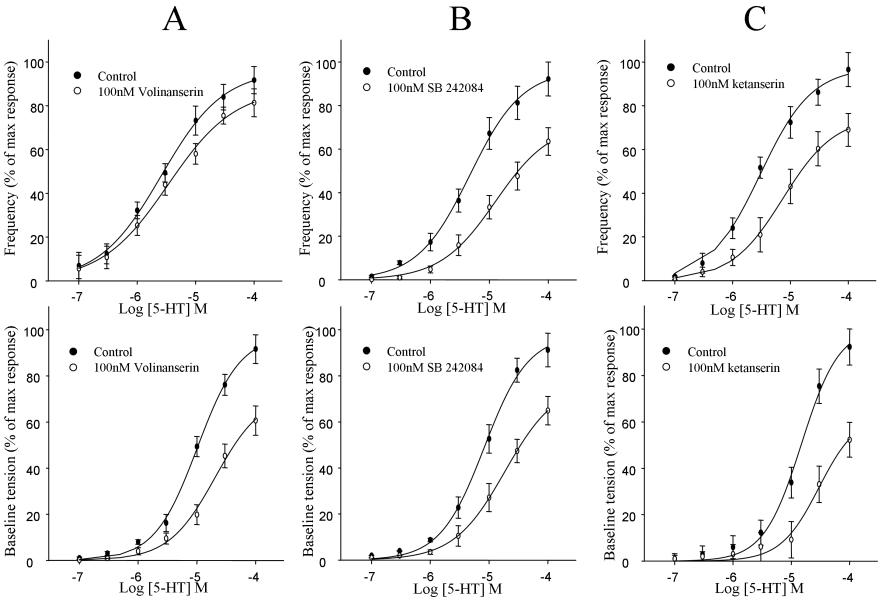


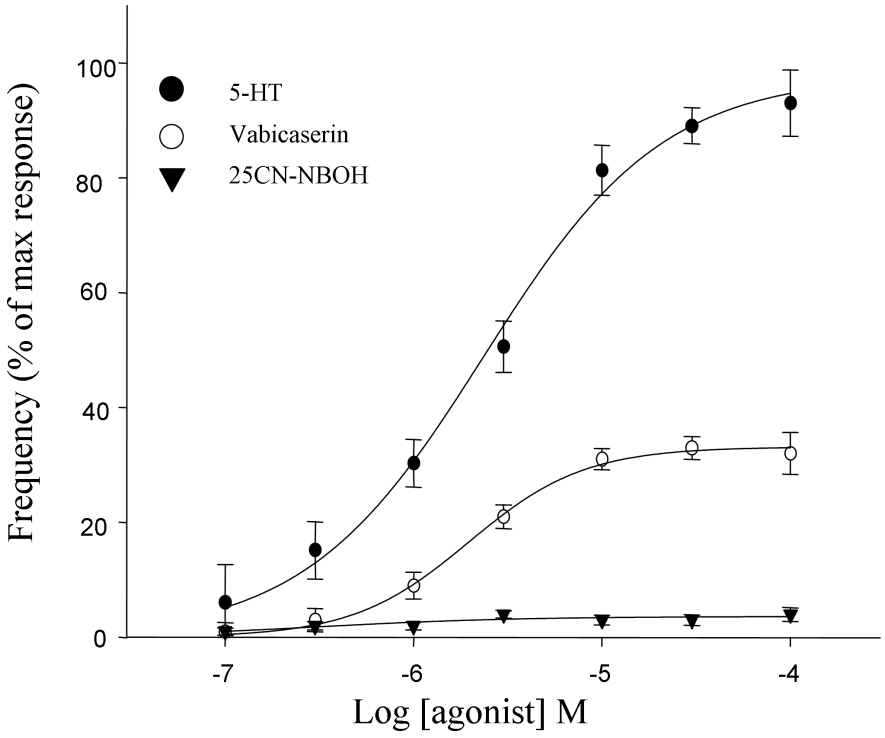

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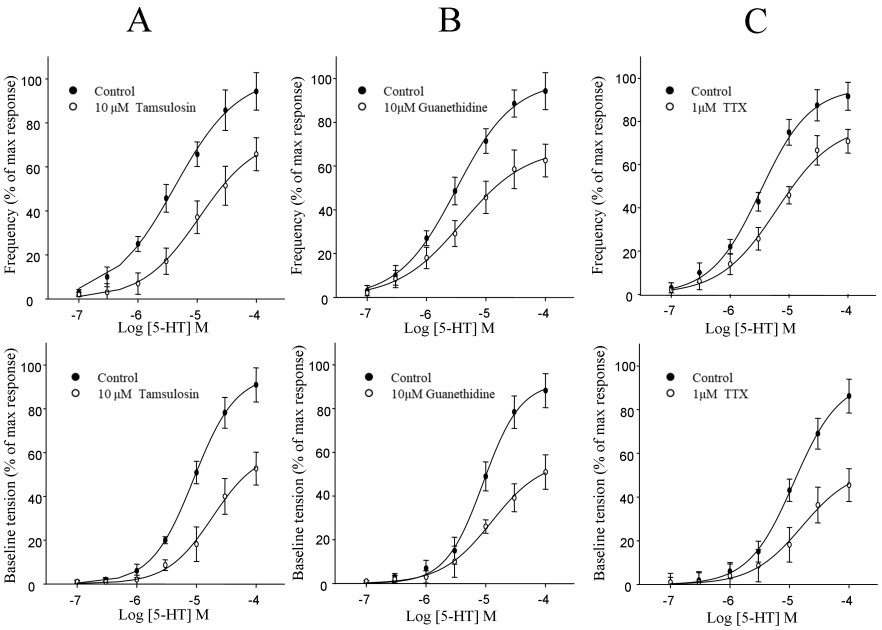


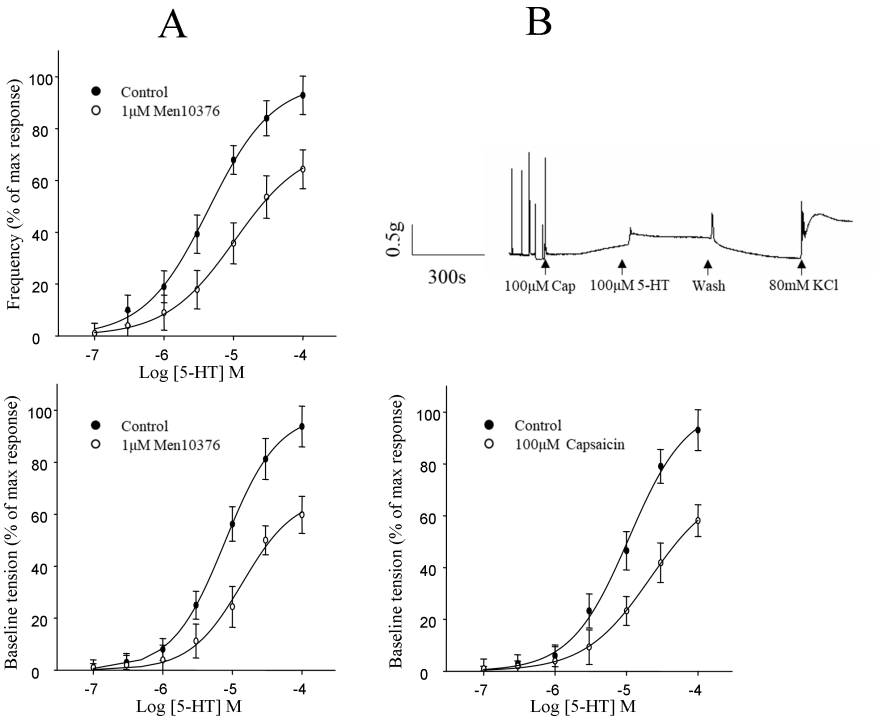

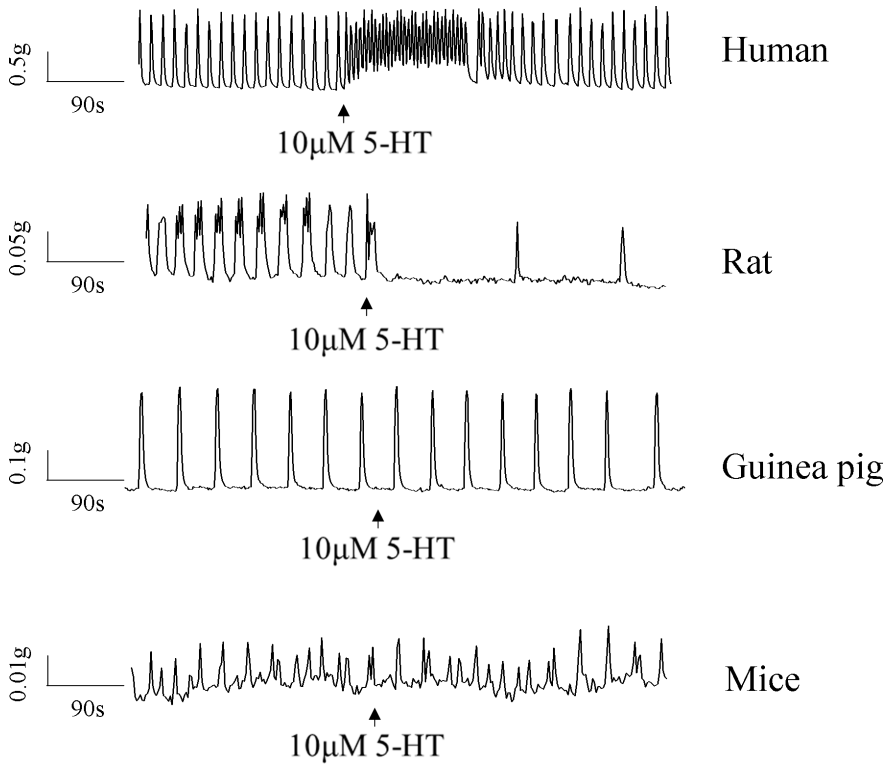


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