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Published in:
European Journal of Pharmacology

DOI:
10.1016/j.ejphar.2021.174024

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Recommended citation(APA):
Lim, I., Christiansen, C., & Chess-Williams, R. (2021). 17β-estradiol and ureteral contractility: A role for the G protein-coupled estrogen receptor. *European Journal of Pharmacology*, *899*, Article 174024. https://doi.org/10.1016/j.ejphar.2021.174024

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Download date: 11 Aug 2024

1 **17β-estradiol and ureteral contractility: A role for the G protein-coupled estrogen**
2 **receptor**
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1 **Abstract**

2 The aim of this study was to investigate the unknown effects of 17β-estradiol (E2) on ureteral
3 contractility and the receptor and mechanisms involved. By utilising isolated porcine distal
4 ureteral strips, we observed that E2 (30 – 300μM) and a G protein-coupled estrogen receptor
5 specific agonist G-1 (30μM) both increased the frequency of phasic contractions of the ureter
6 ($P<0.05$). E2 also decreased the maximum amplitude of these contractions ($P<0.05$). The G
7 protein-coupled estrogen receptor specific antagonist G-36 (10μM) reversed E2 enhancement
8 effects on frequency, but did not alter its effects on maximum amplitude of contractile
9 responses. Additionally, it was observed that the effects of E2 were unaltered by removing
10 the urothelium, inhibiting nitric oxide and prostaglandin production or preventing neuronal
11 conduction. In the presence of a potassium channel blocker, 4-aminopyridine (10μM), the
12 effects of E2 on frequency were prevented. This finding suggests that G protein-coupled
13 estrogen receptor mediates the increase in frequency of ureteral phasic contractions induced
14 by E2 via activation of potassium channels, while E2 alters the amplitude of these
15 contractions through an unknown mechanism.

16

17 **Keywords:**

18 Estradiol; G-protein coupled receptor; Phasic contraction; Porcine; Smooth muscle
19 contraction; Ureter

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1 **1. Introduction**

2 Urolithiasis is a common condition that affects up to 10% of the worldwide population and its
3 prevalence and healthcare burden cost remains on the rise (Abufaraj et al., 2020). Risk factors
4 including low fluid intake, high-salt low-fibre diet and obesity have been linked to increased
5 risk of urolithiasis (Littlejohns et al., 2020). There is also an increased number of urolithiasis
6 clinical presentations among women who had two or more pregnancies or had used female
7 hormone therapy (estrogen and progesterone) (Abufaraj et al., 2020). However, there are also
8 studies that have reported a potential protective role of estrogen against stone lodgement as
9 females are found have a lower risk compared to males (Heller et al., 2002; Ozsoy et al.,
10 2019) and estrogen-deprived post-menopausal women have been shown to have a higher risk
11 of stone formation (Prochaska et al., 2018).
12
13 17β-estradiol (E2), the predominant circulating estrogen in humans, is known classically to
14 activate nuclear estrogen receptors which are responsible mainly for transcriptional events
15 within the cell (Fuentes and Silveyra, 2019). More recently, E2 has also been reported to
16 engage in rapid non-genomic signalling events and has been implicated important for a
17 number of cellular functions (Barton, 2012). This non-genomic activity is thought to be
18 initiated by E2 binding to a membrane receptor, the G-protein coupled estrogen receptor (also
19 known as GPR30) leading to modulation of many signalling pathways and also ultimately
20 altered gene expression following long-term exposure (Jacenik and Krajewska, 2020). G
21 protein-coupled estrogen receptor expression has been found in many tissues of the human
22 body including the breasts, ovaries, uterus, testis, prostate, intestine, smooth and skeletal
23 muscles (Jacenik and Krajewska, 2020).
24

1 Investigations on the functional role of the G protein-coupled estrogen receptor
2 pharmacologically was possible with the development of highly selective agonist G-1 (Kd =
3 10nM) which was shown to not bind to the nuclear estrogen receptors at concentrations up to
4 10μM (Dennis et al., 2011). Subsequently, a highly selective antagonist for this receptor was
5 also identified, namely, G-36, with IC_{50} value of 112nM (Dennis et al., 2011). There is
6 compelling evidence that G protein-coupled estrogen receptor mediates vasodilatory effects
7 of E2 in the renal (Chang et al., 2019) and coronary (Masood et al., 2010; Meyer et al., 2010)
8 vasculature via stimulation of nitric oxide (NO), hydrogen sulfide and prostaglandin release,
9 alongside suppression of calcium sensitivity. In the gastrointestinal system, similar effects of
10 G protein-coupled estrogen receptor activation are observed, inducing relaxation of the rat
11 colon (Tang et al., 2015), human gall bladder (Lee et al., 2016) and the porcine lower
12 oesophageal sphincter (Tsai et al., 2018). In the urinary tract, there is evidence of a functional
13 role for this receptor where its specific agonist G-1 decreases human bladder urothelial cell
14 proliferation (Luo and Liu, 2020). The role of G protein-coupled estrogen receptor in smooth
15 muscle contraction within the urinary tract is sparse but a previous study on the isolated rat
16 detrusor has shown that E2 (30μM) decreases contractile responses induced by electrical field
17 stimulation, carbachol and KCl (Valeri et al., 2009).
18
19 The pathophysiology of urolithiasis has not been clearly elucidated. The formation of stones
20 is known to predominantly occur in the kidney due to calcification. While smaller stones can
21 pass to the bladder spontaneously with urine for expulsion, larger stones frequently lodge in
22 the ureter (Glazer et al., 2020). Patients frequently present clinically with a pressing pain,
23 known as ureteral colic, thought to be caused by the constriction of the ureteral tube resulting
24 in elevated intraureteral pressure (Campschroer et al., 2014). The role of sex hormones in
25 regulating ureteral motility has not been investigated, but is of great interest, considering

1 there is a significantly higher incidence of urolithiasis in males compared to females (Heller
2 et al., 2002; Ozsoy et al., 2019). The aim of this study was to investigate the effects of E2 on
3 agonist-induced contractile responses of the porcine ureter, and to identify the receptor and
4 mechanism through which these effects are mediated.
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1 **2. Materials and Methods**

2 ***2.1 Tissue specimen origin and preparation***

3 Female pig urinary bladders, with ureters attached, from 6-month old female Landrace pigs
4 were obtained from a local abattoir and immediately immersed in ice-cold Krebs-bicarbonate
5 solution (4°C) composed of NaCl (188.4 mM), $NaHCO_3$ (24.9 mM), glucose (11.7 mM),
6 $CaCl_2$ (1.9 mM), $MgSO_4$ (1.2 mM) and KH_2PO_4 (1.2 mM) and transported to the laboratory.
7 The distal segment of the ureter, determined as the 4cm just before entering the bladder, were
8 isolated and dissected into 4mm long tissue strip sections. Tissue strips from the distal ureter
9 were examined, as this is the most common site for urinary stone lodgement (El-Barky et al.,
10 2014). The mucosa was left intact with the smooth muscle strips in all experiments, except
11 when investigating its possible modulatory effects.
12
13 Tissue strips were mounted longitudinally under approximately 1.5 g tension in 8 ml EZ-Bath
14 organ baths (Global Towns Microtechnology, Sarasota, FL, USA) containing Krebs-
15 bicarbonate solution, maintained at 37ºC and continuously gassed with 95% O_2 and 5% CO_2
16 at pH 7.4. All tissue strips were equilibrated for 1 hour (washout with fresh solution every 15
17 mins), before addition of any drug. The isometric tension developed by the tissues was
18 recorded via a Powerlab recording system and Labchart software (ADInstruments, Castle
19 Hill, NSW, Australia).
20

21 ***2.2 Effects of E2 on 5-HT-induced contractility***

22 Our previous studies on the porcine distal ureter have shown that it is not possible to obtain
23 reproducible concentration-response curves due to tachyphylaxis (Lim et al., 2018, 2020).
24 Thus, only one concentration-response curve to 5-HT (59nM – 590µM) was obtained in each
25 tissue. Previous studies from our group also showed that responses to 5-HT in tissues from

1 animals of this specific age group were most reproducible and consistent, in comparison to
2 other agonists including α_1-adrenoceptor agonist phenylephrine or muscarinic receptor
3 agonist carbachol (Lim et al., 2020).
4
5 The short-term effects of E2 (1µM - 100µM) on ureteral contractility were examined by
6 pairing adjacent tissue strips from the same ureter, where one acted as the control and the
7 other incubated with E2 (1µM, 10µM, 30µM, or 100µM) for 25 mins before the addition of
8 increasing cumulative concentrations of 5-HT every 5 mins. To investigate potential
9 relaxatory effects of E2 (100µM), tissue strips were pre-contracted with KCl (80mM),
10 followed by the addition of E2 (100µM) or isoprenaline (10µM), as a positive control, to
11 investigate the possible presence of a relaxant response.
12
13 ***2.3 Mediation of E2 effects on ureteral contractility***
14 Our next set of experiments were performed to investigate the receptor subtype through
15 which E2 mediates its effects in the ureter. For the antagonist experiments, we paired tissue
16 strips from the same ureter and incubated all tissues with either the G protein-coupled
17 estrogen receptor antagonist G-36 (10µM) or the nuclear estrogen receptor antagonist
18 tamoxifen (1µM) for 30 mins. One of the two paired strips was also simultaneously incubated
19 with E2 (100µM) for 25 mins and concentration-response curves to 5-HT were then obtained
20 in all tissues. Preliminary experiments were performed to confirm that these antagonists alone
21 do not affect contractile responses to 5-HT (data not shown). Additionally, we also
22 investigated the effects of the G protein-coupled estrogen receptor selective agonist, G-1
23 (30µM), by incubating the drug with tissues for 25 mins before observing its effects on 5-HT-
24 induced contractility.
25

1 ***2.4 The effects of urothelium, nerve conduction, NO, prostaglandin and potassium***
2 ***channels on E2-induced effects***
3 To investigate the influence of the urothelium on E2 effects, we examined 5-HT-induced
4 responses in the absence or presence of E2 (100μM) in pairs of ureteral strips with the
5 urothelium and lamina propria removed. The involvement of nerve conduction, NO,
6 prostaglandins and potassium channels in the E2 effects were also investigated using the
7 neurotoxin tetrodotoxin (TTX, 1μM), the NO synthase inhibitor Nω-Nitro-L-arginine (L-
8 NNA, 10μM), the cyclo-oxygenase inhibitor indomethacin (10μM) and the K^+ channel
9 blocker 4-aminopyridine (3mM). These drugs were added to the organ bath 30 mins before
10 examining 5-HT concentration response curves in the absence or presence of E2 (100μM).
11 Preliminary experiments and previous published work indicate that these agents alone do not
12 have any effect on 5-HT-induced contractile response in the tissues (Lim et al., 2020).
13
14 ***2.5 Data analysis***
15 In response to the agonist 5-HT, isolated ureteral strips developed bursts of phasic contractile
16 activity (Figs 1A and 1B). These contractile responses appeared to increase in amplitude and
17 frequency in a concentration-dependent manner and were expressed as maximum amplitude
18 (grams tension developed per gram tissue weights, g / g) and frequency (contractions per
19 min) in concentration-response curves. Due to the nature of these phasic contractions, area
20 under the curve (AUC) by weight (gs g^{-1}) was calculated to determine the overall contractile
21 responses of the ureter, accounting for changes in both amplitude and frequency of
22 contractions (Fig 1C). The frequency and AUC were measured over the 5 mins that each
23 concentration of 5-HT was present in the bath.
24

1 GraphPad Prism software (GraphPad, San Diego, CA, USA) was used to perform statistical
2 analysis and graphical representation. All data were expressed as mean ± S.E.M. of 'n'
3 preparations, where 'n' is the number of animals. Non-linear regression analysis of the
4 concentration-response curves was used to determine the potency of the agonists (pEC50, the
5 log of the concentration of a drug that produces half of the maximal response). Mean pEC50
6 values and concentration-response curves were compared using two-tailed, paired or unpaired
7 Student's *t*-tests as appropriate, where $P < 0.05$ was considered statistically significant.
8
9 ***2.6 Drugs and chemicals used***
10 Chemicals were of analytical grade and purchased from Sigma-Aldrich (Castle Hill, NSW,
11 Australia). 17β-estradiol (E2), Nω-Nitro-L-arginine (L-NNA), indomethacin, tetrodotoxin
12 were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia), 5-hydroxytryptamine
13 hydrochloride (5-HT) was obtained from Abcam (Melbourne, VIC, Australia), and G protein-
14 coupled estrogen receptor agonist G-1, G protein-coupled estrogen receptor antagonist G-36
15 and tamoxifen were obtained from Cayman Chemical (Redfern, NSW, Australia). All drugs
16 were dissolved in distilled H_2O except 17β-estradiol, G-36, G-1 and tamoxifen which were
17 all dissolved in DMSO where the final concentration in bath amounted to less than 0.5%
18 DMSO. Preliminary experiments confirmed that this concentration of DMSO (0.5%) did not
19 affect tissue responses (data not shown).
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1 **3. Results**

2 All porcine ureteral strips (mean weight, 31mg ± 0.2mg, n = 154) were allowed to equilibrate
3 to a passive tension of 1.56g ± 0.05g. During the equilibration period, only a few tissue strips
4 (10 out of 154 tissues, 6.5%) developed spontaneous contractions while the other tissues
5 remained quiescent in the absence of agonists. When quiescent tissues were subjected to
6 increasing concentrations of 5-HT, they developed bursts of phasic contractions (Figs 1A and
7 1B). Increasing concentrations of 5-HT induced increases in frequency and maximum
8 amplitude of phasic activity in a concentration-dependent manner.
9

10 ***3.1 Effects of E2 on 5-HT and KCl-induced contractile responses***

11 Upon the addition of E2 (1 - 100μM), the tissue baseline tension was unaffected. The pEC50
12 values of 5-HT in the porcine distal ureteral strips were also unaffected in the presence of E2
13 (1μM, 10μM, 30μM, 100μM) compared to control tissues (n=6 for each E2 concentration,
14 unpaired Student's *t*-tests). Higher concentrations of E2 (30μM and 100μM) increased the
15 frequency of the phasic contractions significantly (Figs 2C and 2E) while lower
16 concentrations did not exert any effect on the frequency (1μM, data not shown, n=6, unpaired
17 Student's *t*-tests; 10μM, Fig 2A). E2 (10μM, 30μM, 100μM) significantly reduced the
18 maximum amplitude of the phasic contractile activity in the tissue strips (Figs 2B, 2D and 2F)
19 while at 1μM, did not produce any effect on the amplitude of the contractile response (data
20 not shown, n=6, unpaired Student's *t*-tests). Overall contractility of the ureter expressed as
21 AUC, decreased in the presence of E2 (100μM, Fig 1C).
22

23 Ureteral tissue strips generated tonic contractions upon addition of a high concentration of
24 KCl (80mM). Following the generation of a sustained tonic contraction, addition of E2
25 (100μM) did not alter the tension of the tissue strips while the addition of isoprenaline

1 (10µM) was always capable of relaxing the tissue strips back to baseline tension (data not
2 shown, n=7).
3
4 ***3.2 Role of G protein-coupled estrogen receptor in E2 effects on ureteral contractility***
5 In the presence of the selective estrogen nuclear receptor modulator tamoxifen (1µM), the
6 effects of E2 (100µM) observed previously were unchanged where 5-HT pEC50 values
7 remained the same, frequency increased and maximum amplitude of 5-HT-induced
8 contractions were decreased in the presence of the hormone (Tables 1 and 2). In contrast, the
9 G protein-coupled estrogen receptor selective antagonist G-36 (10µM) abolished the
10 increased frequency of phasic contractions induced by E2 (Fig 3A), but did not alter the E2
11 effects on maximum amplitude (Fig 3B) and general contractility expressed as AUC also was
12 significantly lower (Fig 3C).
13
14 Incubation of ureteral strips with the G protein-coupled estrogen receptor selective agonist G-
15 1 (30µM) mimicked the E2-enhancing effects on the frequency of 5-HT induced contractions
16 (Fig 3D). However, unlike E2, G-1 failed to alter the maximum amplitude of the phasic
17 contractions (Fig 3E), and subsequently resulted in an increase in contractile response
18 expressed as AUC (Fig 3F).
19
20 ***3.3 Mechanisms involved in E2 effects on ureteral contractility***
21 To determine if the E2 effects were mediated via the urothelium, we examined 5-HT
22 concentration-response curves in the absence and presence of E2 (100µM) in ureteral smooth
23 muscle strips denuded of urothelium and lamina propria. The pEC50 values of 5-HT were
24 similar, frequency of phasic contractions increased (Fig 5A), maximum amplitude decreased

1 (Fig 5B) in the presence of E2 (100μM), and AUC attenuated (Fig 5C) as observed in tissues
2 with intact urothelium and lamina propria from Fig 1.
3
4 In the presence of the NO synthase inhibitor L-NNA (100μM) and cyclooxygenase inhibitor
5 indomethacin (10μM), the effects of E2 previously observed were also unaffected, where
6 pEC50 values remained unchanged, frequency of contractions increased (Table 1) and
7 maximum amplitude of contractions attenuated (Table 2). Decrease in contractile responses
8 to AUC were also similar to that observed in Fig 1C (data not shown). In the presence of the
9 non-specific potassium channel blocker 4-aminopyridine (10μM), the effects of E2 on the
10 frequency of phasic contractions were abolished (Fig 4D), but the E2 effects on maximum
11 amplitude were unchanged (Fig 4E). The decrease in contractility expressed as AUC by E2
12 was also present (Fig 4F).
13
14 **Table 1**. ***E2 (100μM) effects on the potency (pEC50) and maximum frequency values for***
15 ***5-HT-induced ureteral contractile responses in the presence of tamoxifen (1μM), L-NNA***
16 ***(10μM), indomethacin (10μM), and tetrodotoxin (1μM).*** Results presented as mean ±
17 S.E.M. of 'n' preparations (unpaired Student's *t*-tests, [a] $P < 0.05$ vs control)

	pEC50 values		**Maximum Frequency (cpm)**		**n**
	Control	E2	Control	E2	
Tamoxifen (1μM)	5.54 ± 0.49	5.60 ± 0.42	10.2 ± 1.3	18.1 ± 2.1 [a]	5
L-NNA (100μM)	5.46 ± 0.21	5.61 ± 0.50	9.3 ± 0.9	17.3 ± 1.2 [a]	5
Indomethacin (10μM)	5.73 ± 0.23	5.46 ± 0.20	11.4 ± 0.8	16.2 ± 0.9 [a]	6
Tetrodotoxin (1μM)	5.64 ± 0.26	5.82 ± 0.35	11. 2 ± 0.6	18.3 ± 1.1 [a]	6

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19

1 **Table 2**. ***E2 (100µM) effects on the potency (pEC50) and maximum amplitude values for***
2 ***5-HT induced ureteral contractile responses in the presence of tamoxifen (1µM), L-NNA***
3 ***(10µM), indomethacin (10µM), and tetrodotoxin (1µM).*** Results presented as mean ±
4 S.E.M. of 'n' preparations (unpaired Student's *t*-test, [a] $P < 0.05$ vs control.

	pEC50 values		**Maximum Amplitude (g /g)**		**n**
	Control	E2	Control	E2	
Tamoxifen (1µM)	6.42 ± 0.91	6.12 ± 0.82	294.1 ± 21.1	92.9 ± 10.0 [a]	5
L-NNA (10µM)	6.14 ± 0.51	5.91 + 0.91	283.2 ± 13.1	101.1 ± 9.1 [a]	5
Indomethacin (10µM)	5.98 ± 0.42	6.32 ± 1.99	272.1 ± 32.5	103.7 ± 16.8 [a]	6
Tetrodotoxin (1µM)	6.20 ± 0.67	5.91 ± 0.82	278.3 ± 15.1	94.9 ± 11.3 [a]	6

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1 **4. Discussion**

2 The role of E2 in the urinary tract is not fully elucidated and particularly in the ureter, its role
3 is unknown. In this study, the isolated porcine ureter, which have been proposed to be similar
4 to healthy human ureter pharmacologically and physiologically (Lim et al., 2020), was
5 treated with E2 at various concentrations *in vitro* to determine if the hormone has a role in
6 smooth muscle contraction in this tissue.

7

8 The current results show that E2 at concentrations of 30μM and above, alters 5-HT-induced
9 phasic contractile responses of the porcine ureter in two different aspects: increase in
10 frequency of contractions and attenuation of maximum amplitude responses. The contractile
11 responses of the ureter has been shown to have differing characteristics where bursts of
12 phasic contractions develop in response to agonist, compared to other parts of the urinary
13 tract like the bladder (Kang et al., 2015) where tonic contractions are usually exhibited.
14 However, estrogen has previously been shown to exert a similar inhibitory effect on the
15 amplitude of carbachol-induced contractions in the isolated human (Elliott et al., 1992), pig
16 (Dambros et al., 2004) and rat (Elliott et al., 1992; Valeri et al., 2009) detrusor smooth
17 muscle. Additionally, pre-treatment with estrogen in the rat *in vivo* was reported to reduce the
18 maximum contractile response of the detrusor smooth muscle to acetylcholine and electrical
19 stimulation (Elliott et al., 1992). Contrastingly, mouse pre-treated with estradiol in a separate
20 investigation suggested a decreased in maximum response to methacholine (Ma et al., 2004).
21 However, there was also a decreased response to high-K(+) physiological salt solution, which
22 suggests that this inhibitory effect might not be estrogen receptor-specific (Ma et al., 2004).

23

24 The pathophysiology of ureteral colic is not clearly elucidated, but it is thought that in this
25 condition, there is an increased contractility of the ureteral smooth muscle wall, and

subsequently increased intraureteral pressure (Campschroer et al., 2014). The nature of ureteral contractile responses where bursts of phasic contractions are observed, makes it difficult and complex to investigate. An increase in frequency of ureteral contractions induced by E2 might promote ureteral peristalsis, and therefore can be regarded as a favourable factor to increase spontaneous passage of stones. The decreased amplitude might reduce intraureteral pressure, which has been corelated with reduced afferent nerve activity and pain (Johnson et al., 2016). Additionally, overall contractility of the ureter expressed as AUC suggests E2 decreases the general contractile activity which again, further promotes the notion that E2 might enhance stone passage. This finding might explain the increased number of clinical presentations with colic in women post-menopause and in males compared to females (Heller et al., 2002; Ozsoy et al., 2019; Prochaska et al., 2018).

While it is well established that E2 exerts most of its long-term effects via the nuclear estrogen receptor by mainly altering gene transcription and cellular processes, several factors contradict the involvement of nuclear receptors in this study, including: (i) the rapid effect that E2 exerted on ureteral contractility, which is inconsistent with the time course required for gene transcription (Barton, 2016), (ii) the concentration of E2 required to exert these effects are higher than required for genomic activation (McEwen, 1991), and (iii) the inability of tamoxifen to suppress these effects (Comeglio et al., 2014). Therefore, it was predicted that the effects of E2 were mediated via a membrane-bound receptor. Additionally, the G protein-coupled estrogen receptor has been shown to be involved in smooth muscle contraction and relaxation within the intestine (Jacenik and Krajewska, 2020), oesophagus (Tsai et al., 2018) and also in the detrusor muscle (Valeri et al., 2009). Interestingly, our results show that the G protein-coupled estrogen receptor antagonist G-36 was only able to reverse the E2 enhancement of frequency responses to 5-HT and not the inhibitory effects of

1 E2 on amplitude responses. The G protein-coupled estrogen receptor agonist G-1 mimicked
2 the effects of E2 on the frequency of contraction, confirming that E2 most likely increases
3 frequency of phasic contractions via this receptor, but did not produce any effect on the
4 amplitude of the contractile response, confirming different E2 mechanisms on this parameter.
5 Surprisingly, the E2 effect on amplitude did not appear to be mediated via E2 nuclear
6 receptors either, since the presence of tamoxifen was unable to reverse this. Therefore, the
7 mechanism involved in E2 depressing amplitude remain unknown.
8
9 Previous studies have suggested that G protein-coupled estrogen receptor involvement in
10 smooth muscle activity is mediated via various mechanisms including neurogenic modulation
11 (Li et al., 2016), production of NO (Teoh et al., 2020), prostaglandin release (Huang et al.,
12 2018) and activation of potassium channels (Tsai et al., 2018). Tetrodotoxin, L-NNA and
13 indomethacin did not inhibit the effects of E2 which demonstrates that in the porcine distal
14 ureter, E2-related effects are not attributed to neurotransmitter release, NO or prostaglandin
15 production. In a previous study on the isolated human detrusor, E2 was suggested to reduce
16 spontaneous phasic contractions via the activation of potassium channels (Hristov et al.,
17 2017). In the ureter, our results show that blockade of potassium channels by 4-
18 aminopyridine reversed the E2 effects on the frequency of ureteral phasic contractions, which
19 suggests that this increase in frequency is associated with the opening of potassium channels.
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5. Conclusions

Our study is the first to demonstrate that E2 applied *in vitro* alters the contractile responses of the porcine ureter by increasing the frequency of phasic contractions, mediated by G protein-coupled estrogen receptor and also by decreasing the amplitude of these contractions via an unknown mechanism. Our results suggest that the stimulated G protein-coupled estrogen receptor might activate the opening of potassium channels in the ureter to exert an increase in frequency.

Funding Source: Iris Lim received a Faculty Early Career Research Grant (Bond University) which supported the experimental work for this study. The funding source was not involved in the study design, data collection and analysis, report writing and decision to submit the article for publication.

Ethical Statement: The animal tissues utilised in this study were obtained from an abattoir after slaughter for food and were considered waste and therefore, did not require ethical approval by the institutional committee.

Conflict of Interest: None to report or disclose.

References

Abufaraj, M., Xu, T., Cao, C., Waldhoer, T., Seitz, C., D'Andrea, D., Siyam, A., Tarawneh, R., Fajkovic, H., Schernhammer, E., Yang, L., & Shariat, S. F. (2020, Sep 5). Prevalence and Trends in Kidney Stone Among Adults in the USA: Analyses of National Health and Nutrition Examination Survey 2007-2018 Data. *Eur Urol Focus*. https://doi.org/10.1016/j.euf.2020.08.011

Barton, M. (2012, Aug). Position paper: The membrane estrogen receptor GPER--Clues and questions. *Steroids, 77*(10), 935-942. https://doi.org/10.1016/j.steroids.2012.04.001

Barton, M. (2016, Jul). Not lost in translation: Emerging clinical importance of the G protein-coupled estrogen receptor GPER. *Steroids, 111*, 37-45. https://doi.org/10.1016/j.steroids.2016.02.016

Campschroer, T., Zhu, Y., Duijvesz, D., Grobbee, D. E., & Lock, M. T. (2014, Apr 2). Alpha-blockers as medical expulsive therapy for ureteral stones. *Cochrane Database Syst Rev*(4), CD008509. https://doi.org/10.1002/14651858.CD008509.pub2

Chang, Y. C., Han, Z. W., Zhang, Y., Zhang, Y., Feng, Z. Y., Chen, L., Li, X. R., Li, L., & Si, J. Q. (2019, Apr). G protein-coupled estrogen receptor activation improves contractile and diastolic functions in rat renal interlobular artery to protect against renal ischemia reperfusion injury. *Biomedicine & Pharmacotherapy, 112*. https://doi.org/ARTN 108666
10.1016/j.biopha.2019.108666

Comeglio, P., Morelli, A., Cellai, I., Vignozzi, L., Sarchielli, E., Filippi, S., Maneschi, E., Corcetto, F., Corno, C., Gacci, M., Vannelli, G. B., & Maggi, M. (2014, Jan). Opposite effects of tamoxifen on metabolic syndrome-induced bladder and prostate alterations: a role for GPR30/GPER? *Prostate, 74*(1), 10-28. https://doi.org/10.1002/pros.22723

Dambros, M., van Koeveringe, G. A., Bast, A., & van Kerrebroeck, P. E. (2004, Oct). Relaxant effects of estradiol through non-genomic pathways in male and female pig bladder smooth muscle. *Pharmacology, 72*(2), 121-127. https://doi.org/10.1159/000080184

Dennis, M. K., Field, A. S., Burai, R., Ramesh, C., Petrie, W. K., Bologa, C. G., Oprea, T. I., Yamaguchi, Y., Hayashi, S., Sklar, L. A., Hathaway, H. J., Arterburn, J. B., & Prossnitz, E. R. (2011, Nov). Identification of a GPER/GPR30 antagonist with improved estrogen receptor counterselectivity. *J Steroid Biochem Mol Biol, 127*(3-5), 358-366. https://doi.org/10.1016/j.jsbmb.2011.07.002

El-Barky, E., Ali, Y., Sahsah, M., Terra, A. A., & Kehinde, E. O. (2014, Feb). Site of impaction of ureteric calculi requiring surgical intervention. *Urolithiasis, 42*(1), 67-73. https://doi.org/10.1007/s00240-013-0605-4

Elliott, R. A., Castleden, C. M., & Miodrag, A. (1992, Nov). The effect of in vivo oestrogen pretreatment on the contractile response of rat isolated detrusor muscle. *Br J Pharmacol, 107*(3), 766-770. https://doi.org/10.1111/j.1476-5381.1992.tb14521.x

1
2 Elliott, R. A., Castleden, C. M., Miodrag, A., & Kirwan, P. (1992). The direct effects of
3 diethylstilboestrol and nifedipine on the contractile responses of isolated human and
4 rat detrusor muscles. *Eur J Clin Pharmacol, 43*(2), 149-155.
5 https://doi.org/10.1007/BF01740662
6
7 Fuentes, N., & Silveyra, P. (2019). Estrogen receptor signaling mechanisms. *Adv Protein*
8 *Chem Struct Biol, 116*, 135-170. https://doi.org/10.1016/bs.apcsb.2019.01.001
9
10 Glazer, K., Brea, I. J., & Vaitla, P. (2020). Ureterolithiasis. In *StatPearls*.
11 https://www.ncbi.nlm.nih.gov/pubmed/32809509
12
13 Heller, H. J., Sakhaee, K., Moe, O. W., & Pak, C. Y. (2002, Nov). Etiological role of
14 estrogen status in renal stone formation. *J Urol, 168*(5), 1923-1927.
15 https://doi.org/10.1097/01.ju.0000033907.21910.be
16
17 Hristov, K. L., Parajuli, S. P., Provence, A., Rovner, E. S., & Petkov, G. V. (2017, Jul).
18 Nongenomic modulation of the large conductance voltage- and Ca(2+)-activated K(+)
19 channels by estrogen: A novel regulatory mechanism in human detrusor smooth
20 muscle. *Physiol Rep, 5*(14). https://doi.org/10.14814/phy2.13351
21
22 Huang, N., Wang, C., Zhang, N., Mao, W., Liu, B., Shen, Y., Gao, Y., Zhao, Y., & Cao, J.
23 (2018, Jan 5). Effect of estrogen on prostaglandin synthetase in bovine oviduct
24 smooth muscle. *Eur J Pharmacol, 818*, 287-293.
25 https://doi.org/10.1016/j.ejphar.2017.10.058
26
27 Jacenik, D., & Krajewska, W. M. (2020). Significance of G Protein-Coupled Estrogen
28 Receptor in the Pathophysiology of Irritable Bowel Syndrome, Inflammatory Bowel
29 Diseases and Colorectal Cancer. *Front Endocrinol (Lausanne), 11*, 390.
30 https://doi.org/10.3389/fendo.2020.00390
31
32 Johnson, L. J., Davenport, D., & Venkatesh, R. (2016, Apr). Effects of Alpha-Blockade on
33 Ureteral Peristalsis and Intrapelvic Pressure in an In Vivo Stented Porcine Model. *J*
34 *Endourol, 30*(4), 417-421. https://doi.org/10.1089/end.2015.0251
35
36 Kang, S. H., McDermott, C., Farr, S., & Chess-Williams, R. (2015, Jul). Enhanced urothelial
37 ATP release and contraction following intravesical treatment with the cytotoxic drug,
38 doxorubicin. *Naunyn Schmiedebergs Arch Pharmacol, 388*(7), 773-780.
39 https://doi.org/10.1007/s00210-015-1097-2
40
41 Lee, M. C., Yang, Y. C., Chen, Y. C., Chang, B. S., Li, Y. C., & Huang, S. C. (2016, Apr-
42 Jun). Estrogen and G protein-coupled estrogen receptor agonist G-1 cause relaxation
43 of human gallbladder. *Ci Ji Yi Xue Za Zhi, 28*(2), 54-58.
44 https://doi.org/10.1016/j.tcmj.2016.03.004
45
46 Li, Y., Xu, J., Jiang, F., Jiang, Z., Liu, C., Li, L., Luo, Y., Lu, R., Mu, Y., Liu, Y., & Xue, B.
47 (2016, Mar). G protein-coupled estrogen receptor is involved in modulating colonic
48 motor function via nitric oxide release in C57BL/6 female mice. *Neurogastroenterol*
49 *Motil, 28*(3), 432-442. https://doi.org/10.1111/nmo.12743
50

1 Lim, I., Chess-Williams, R., & Sellers, D. (2018, Apr). Altered ureteral contractility with
2 ageing: Role of the rho-kinase pathway. *Mech Ageing Dev, 171*, 31-36.
3 https://doi.org/10.1016/j.mad.2018.03.004
4
5 Lim, I., Chess-Williams, R., & Sellers, D. (2020, Mar - Apr). A porcine model of ureteral
6 contractile activity: Influences of age, tissue orientation, region, urothelium, COX and
7 NO. *J Pharmacol Toxicol Methods, 102*, 106661.
8 https://doi.org/10.1016/j.vascn.2019.106661
9
10 Littlejohns, T. J., Neal, N. L., Bradbury, K. E., Heers, H., Allen, N. E., & Turney, B. W.
11 (2020, Jul 15). Fluid Intake and Dietary Factors and the Risk of Incident Kidney
12 Stones in UK Biobank: A Population-based Prospective Cohort Study. *Eur Urol*
13 *Focus, 6*(4), 752-761. https://doi.org/10.1016/j.euf.2019.05.002
14
15 Luo, J., & Liu, D. M. (2020, Mar 31). Does GPER Really Function as a G Protein-Coupled
16 Estrogen Receptor in vivo? *Frontiers in Endocrinology, 11*. https://doi.org/ARTN 148
17 10.3389/fendo.2020.00148
18
19 Ma, S., Story, M. E., & Pennefather, J. N. (2004, Mar 8). Muscarinic receptors mediating
20 contraction of female mouse urinary bladder: effects of oestrogen. *European Journal*
21 *of Pharmacology, 487*(1-3), 205-211. https://doi.org/10.1016/j.ejphar.2004.01.022
22
23 Masood, D. E., Roach, E. C., Beauregard, K. G., & Khalil, R. A. (2010, Oct). Impact of sex
24 hormone metabolism on the vascular effects of menopausal hormone therapy in
25 cardiovascular disease. *Curr Drug Metab, 11*(8), 693-714.
26 https://doi.org/10.2174/138920010794233477
27
28 McEwen, B. S. (1991, Apr). Non-genomic and genomic effects of steroids on neural activity.
29 *Trends Pharmacol Sci, 12*(4), 141-147. https://doi.org/10.1016/0165-6147(91)90531-
30 v
31
32 Meyer, M. R., Baretella, O., Prossnitz, E. R., & Barton, M. (2010). Dilation of epicardial
33 coronary arteries by the G protein-coupled estrogen receptor agonists G-1 and ICI
34 182,780. *Pharmacology, 86*(1), 58-64. https://doi.org/10.1159/000315497
35
36 Ozsoy, M., Somani, B., Seitz, C., Veser, J., & Kallidonis, P. (2019, May). Sex differences in
37 the therapy of kidney and ureteral stones. *Curr Opin Urol, 29*(3), 261-266.
38 https://doi.org/10.1097/MOU.0000000000000604
39
40 Prochaska, M., Taylor, E. N., & Curhan, G. (2018, Oct). Menopause and Risk of Kidney
41 Stones. *J Urol, 200*(4), 823-828. https://doi.org/10.1016/j.juro.2018.04.080
42
43 Tang, Y. R., Yang, W. W., Wang, Y., Gong, Y. Y., Jiang, L. Q., & Lin, L. (2015). Estrogen
44 regulates the expression of small-conductance Ca-activated K+ channels in colonic
45 smooth muscle cells. *Digestion, 91*(3), 187-196. https://doi.org/10.1159/000371544
46
47 Teoh, J. P., Li, X., Simoncini, T., Zhu, D., & Fu, X. (2020, Oct). Estrogen-Mediated Gaseous
48 Signaling Molecules in Cardiovascular Disease. *Trends Endocrinol Metab, 31*(10),
49 773-784. https://doi.org/10.1016/j.tem.2020.06.001
50

Tsai, C. C., Tey, S. L., Chang, L. C., Su, Y. T., Lin, K. J., & Huang, S. C. (2018, Aug). Estradiol mediates relaxation of porcine lower esophageal sphincter. *Steroids, 136*, 56-62. https://doi.org/10.1016/j.steroids.2018.05.001

Valeri, A., Brain, K. L., Young, J. S., Sgaragli, G., & Pessina, F. (2009, Jul). Effects of 17beta-oestradiol on rat detrusor smooth muscle contractility. *Exp Physiol, 94*(7), 834-846. https://doi.org/10.1113/expphysiol.2009.047118

1 **Figure Legends**

2 **Fig 1.** ***Raw data trace showing bursts of phasic contractions developed by the isolated***
3 ***porcine distal ureter after the addition of 100μM 5-HT (maximum contractile response) in***
4 ***the absence (A) and presence (B) of E2 (100μM) and the corresponding 5-HT***
5 ***concentration response curves expressed as AUC by weight, gs g^{-1} (C).*** Black triangle
6 denotes the timepoint where the agonist was added into the organ bath. Data points in
7 concentration response curves are presented as mean ± S.E.M. of 6 preparations (unpaired
8 Student's *t*-tests, * P < 0.05 vs control).

9 F**ig 2.** ***Concentration-response curves for 5-HT in isolated distal porcine ureter in the***
10 ***absence and presence of E2 (10 μM, A-B; 30 μM, C-D; 100 μM, E-F).*** Responses are
11 expressed as frequency (A, C, and E) and maximum amplitude (B, D and F) expressed as
12 grams developed tension per gram tissue weight. Data points presented as mean ± S.E.M. of
13 6 preparations for each group (unpaired Student's *t*-test, * P < 0.05 vs control).

14 **Fig 3.** ***Concentration response curves for 5-HT in isolated distal porcine ureter in the***
15 ***presence of G-36 (10μM) in the presence and absence of E2 (100μM) (A-C) and in the***
16 ***absence and presence of G-1 (10μM) (D-F).*** Responses are expressed as frequency (A and
17 D), maximum amplitude (B and E) and AUC (C and F) expressed as grams developed tension
18 per gram tissue weight. Data points presented as mean ± S.E.M. of 6 preparations (unpaired
19 Student's *t*-tests, * P < 0.05 vs control).

20 **Fig 4.** ***The effects of E2 (100μM) on 5-HT-induced contractions in isolated distal porcine***
21 ***ureteral strips denuded of urothelium and lamina propria (A-C) and in the presence of 4-***
22 ***aminopyridine (3mM) (D-F).*** Responses are expressed as frequency (A and D), maximum
23 amplitude (B and E) and AUC (C and F) expressed as grams developed tension per gram
24 tissue weight. Data points presented as mean ± S.E.M. of 6 preparations (unpaired Student's
25 *t*-tests, * P < 0.05 vs control).