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Doxazosin modifies serotonin-mediated rabbit urinary bladder contraction

Potential clinical relevance

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Abstract 5-Hydroxytryptamine (5-HT) induces rabbit detrusor contractions via 5-HT_3 receptors. Similarly, 5-HT_4 receptors are known to be present in the human bladder. Doxazosin, a non-selective α_1 antagonist, is used for the symptomatic relief of bladder outflow obstruction. Previous work has shown that doxazosin inhibits 5-HT_2-mediated platelet shape change. Hence, the aim of this study was to assess, using organ baths and autoradiography, whether doxazosin has any 5-HT-inhibiting activity in the rabbit detrusor. Detrusor strips from adult New Zealand White rabbits were placed in organ baths; phenoxybenzamine (10^{-5} M) was added to block alpha-receptors. After KCl responses were assessed, the tissues were exposed to 10^{-3} M 5-HT. Subsequently, the strips were incubated with doxazosin or ondansetron (10^{-5} M; 5-HT_3 antagonist) followed by a further exposure to 5-HT. In some experiments, after the initial 5-HT-induced contractions, the tissues were washed and then re-exposed to 5-HT. These latter experiments acted as controls. Low-resolution autoradiography was performed on detrusor sections to assess the effect of doxazosin on 5-HT binding. These sections were analyzed densitometrically. Doxazosin and ondansetron produced a significant reduction in 5-HT-mediated contractions. Inhibition by doxazosin was in a concentration-dependent manner. Autoradiography demonstrated a significant reduction in [^{3}H]-5-HT binding by doxazosin. Doxazosin significantly inhibits 5-HT-mediated contractions in the rabbit detrusor. This effect appears to be mainly mediated via 5-HT_3 receptor inhibition. Autoradiographic evidence suggests that doxazosin reduces 5-HT binding in the rabbit detrusor. The beneficial effects of doxazosin in bladder outflow obstruction may be due, at least in part, to 5-HT antagonism.

Key words Doxazosin · Serotonin · Rabbit · Urinary bladder

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Introduction

Over the past decade, serotonin (5-hydroxytryptamine, 5-HT) receptor subtypes have been identified in the lower urinary tract of humans and animals [3, 6, 9, 11, 29, 42, 45]. Excitatory effects elicited by 5-HT have been described in both unstimulated and electrically stimulated detrusor strips in the human [11, 24] and rabbit [3, 6]. These actions are thought to be mediated via 5-HT_4 receptors in humans [11] and 5-HT_3 receptors in rabbits [3]. There is also evidence to suggest that 5-HT may be a physiologically and/or pathologically relevant neurotransmitter in the lower urinary tract [24].

Benign prostatic hyperplasia (BPH) is both a common and debilitating disease [4, 26, 30, 41, 43]. Patients with BPH may experience both voiding and irritative symptoms. Doxazosin, like prazosin and terazosin, is an antagonist of α_1-adrenoceptors [14, 33]. This drug can improve both the voiding and irritative symptoms associated with BPH (J.Y. Gillenwater, R.L. Conn, S.G. Chrysant, unpublished work). The improvement in voiding symptoms can be explained by the fact that there is a high density of α_1-adrenoceptors within the smooth muscle of the prostatic adenoma, prostatic capsule, proximal urethra and bladder base [47]. This is relevant because prostatic urethral smooth muscle tone is mainly mediated by α_1-adrenoceptors [22, 23]. Furthermore, doxazosin displays selective affinity for α_1-adrenoceptors within human prostatic tissue and

produces a dose-dependent inhibition of phenylephrine (a selective α_1-adrenoceptor agonist)-induced prostatic contraction [27]. Therefore, this property could account for the improvement in voiding symptoms experienced by patients with BPH taking this drug. More difficult to explain is alleviation of the irritative symptoms. However, doxazosin may also have a central site of action that may contribute to the improvement in the irritative symptoms [20]. In addition, beneficial effects on the irritative symptoms could also be 5-HT-dependent, since in animal models of urological pathology such as bladder instability there is increased local contractility in response to 5-HT [1, 3, 5, 6, 11, 24, 28, 35–38]. This possibility deserves further investigation, because we previously demonstrated that doxazosin inhibits 5-HT-mediated platelet shape change [21].

In the present study, we aimed to determine whether doxazosin exerts any 5-HT-inhibiting activity in the rabbit bladder. We propose that such an effect (by decreasing 5-HT-mediated detrusor contractility) may explain why doxazosin improves the irritative symptoms associated with BPH and possibly other bladder pathology.

Materials and methods

Animals

Male New Zealand White (NZW) rabbits (3 kg) were used in the functional ($n = 6$) and autoradiographic study ($n = 6$). All animals were fed ad libitum with SDS standard plain diet (SDS, Witham, UK) and allowed free access to water.

Functional studies

Following cervical dislocation, detrusor strips ($n = 12$) devoid of urothelium were taken from the anterior wall of the dome. The strips measured approximately $1 \times 1 \times 5$ mm. In all experiments, strips were mounted vertically in 1.5-ml organ baths containing Tyrode's solution with phenoxybenzamine, maintained at 37 °C by a thermoregulated circuit. Phenoxybenzamine (10^{-5} M; non-selective α-adrenoceptor antagonist) was added to the Tyrode's solution in order to inhibit any potential α-adrenergic activity. This is because 5-HT is known to increase the pressure within the bladder in mammals when injected intra-arterially [38]. This action of 5-HT was greatly reduced by the α-receptor antagonist phentolamine [12]. Hence, it was proposed that 5-HT produced its effects via α-receptors [12]. Thus, by adding phenoxybenzamine we insured that any 5-HT-mediated contractions would be via its own specific receptors and not α-receptors. The Tyrode's solution was bubbled with a mixture of 95% O_2 and 5% CO_2, maintaining pH at 7.4. An initial tension of 2 g was applied to the suspended tissue strips. The tension was recorded with a force-displacement transducer (FT-03; Grass Instruments, Quincy, Mass., USA) on a Grass Polygraph (model 7D). All strips were equilibrated for 45 min. At the end of the equilibration period, the strips were challenged with KCl (124 mM). Two reproducible contractions varying in magnitude by less than 10% were consistently obtained. Phenylephrine (10^{-9} to 10^{-4} M) was added to some of the detrusor strips but did not produce any contractions, thereby demonstrating the effective α-adrenoceptor-inhibiting action of phenoxybenzamine (data not shown). 5-HT (10^{-3} M) was subsequently added to the detrusor strips. Due to the tachyphylaxis produced by 5-HT, it was not possible to produce cumulative dose-response curves. Hence, only one concentration of 5-HT was used on each tissue sample. The concentration of 5-HT (10^{-3} M) that produced maximal contraction was used to assess the effect of doxazosin. The strips were then re-washed until the baseline values were obtained. The strips were then re-exposed to 5-HT (10^{-3} M) 30 min later and used as controls ($n = 12$) or were incubated in doxazosin (10^{-4} to 10^{-6} M, $n = 12$ each for the different concentration of doxazosin used) for 30 min and then re-exposed to 5-HT (10^{-3} M). Some strips ($n = 6$), after the initial exposure to 5-HT (10^{-3} M), were incubated in ondansetron (10^{-5} M; 5-HT_3 antagonist) for 30 min and then re-exposed to 5-HT (10^{-3} M).

We also carried out some experiments in the absence of phenoxybenzamine to determine whether there would be a difference in the 5-HT-mediated contractions.

Preparation of tissues for autoradiographs

Following cervical dislocation, the urinary bladders were excised and stored immediately at −70 °C in airtight containers. Due to technical difficulties involved in separating the urothelium from the detrusor, the detrusor blocks with the urothelium intact were dissected and subsequently mounted in AMES OCT embedding compound (BDH Laboratory Supplies, Poole, UK). Transverse sections (10 μmm, $n = 12$) were cut in a cryostat at approximately −20 °C and thaw-mounted onto gelatinized microscope slides. The slides were stored at −70 °C in airtight containers until use.

Autoradiographic studies

Consecutive serial rabbit detrusor sections with the urothelium intact (10 μmm; $n = 12$) were initially pre-incubated in 170 mM TRIS HCl buffer, pH 7.5, for 30 min at 22 °C in order to reduce endogenous transmitter levels. Slides were then transferred to 170 mM TRIS HCl buffer, pH 7.4 (plus 0.01% ascorbic acid, 4 mM $CaCl_2$, 10 μmM pargyline) in the presence of 10 nmol/l [^{3}H]-5-HT (specific binding) obtained from Amersham International. These concentrations were at the approximate K_D values established from previous saturation studies [13]. The degree of non-specific binding was established by incubating adjacent sections ($n = 12$) in the presence of 10 μmmol/l unlabeled 5-HT creatinine sulphate (non-specific binding for [^{3}H]-5-HT was less than 5%; Sigma, UK). Competition studies were performed where [^{3}H]-5-HT binding was determined as above in the presence of doxazosin (10^{-8} to 10^{-6} M; $n = 12$ for each doxazosin concentration). Low-resolution autoradiography was carried out by exposing sections to Hyperfilm 3H (Amersham International) in X-ray cassettes for 12 weeks.

Densitometric analysis was performed using an image system (Model GS-700 Imaging Densitometer; BIO-RAD, Hertfordshire, UK). Binding was finally expressed in terms of radioligand bound (disintegrations per min, dpm) per unit area (millimetres squared), calculated from standard curves generated by ^{3}H microscales (Amersham International) that were co-exposed with tissue sections.

Drugs and solutions

5-HT creatinine sulphate was obtained from Sigma-Aldrich, Poole, Dorset, UK; [^{3}H]-5-HT was obtained from Amersham International, Bucks, UK; doxazosin was obtained from Pfizer, Sandwich, Kent, UK; ondansetron was obtained from Glaxo Wellcome UK. The Tyrode's solution used had the following composition: NaCl 118 mM, KCl 4.0 mM, $NaHCO_3$ 24.0 mM, NaH_2PO_4 0.4 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.8 mM, glucose 6.1 mM, sodium pyruvate 5.0 mM; and the Kreb's transporting solution had a composition of: NaCl 115 mM, $NaHCO_3$ 24.4 mM, KCl 4.0 mM, NaH_2PO_4 0.5 mM, $CaCl_2$ 0.7 mM.

Statistical analysis

All data are given as median and range. Wilcoxon two-tailed tests for paired values were used for the statistical analyses. The principles of laboratory animal care were followed and Home Office approval was sought prior to starting the study.

Results

Functional studies

There were no significant differences in the weights and lengths of smooth muscle strips used in the control and doxazosin-incubated studies. In the control studies, the tissues exposed to 5-HT alone elicited significant contractions that were calculated as a percentage of the initial KCl response (90 ± 5%). Doxazosin significantly inhibited 5-HT-mediated detrusor contractions in a concentration-dependent manner. Doxazosin at 10^{-6} M produced a 12% (9–16%) reduction ($P = 0.02$), 10^{-5} M resulted in 52% (45–57%) reduction ($P = 0.002$) and 10^{-4} M produced a 76% (69–81%) reduction ($P = 0.002$; Fig. 1). Ondansetron also significantly inhibited ($P = 0.002$) 5-HT-mediated detrusor contractions [70% (64–80%) reduction in contraction]. In the absence of phenoxybenzamine, there was no difference in the 5-HT-mediated detrusor contractions (data not shown).

Autoradiography

There was dense binding of [^{3}H]-5-HT to both the detrusor and the urothelium, with, [^{3}H]-5-HT binding significantly greater to the urothelium than the detrusor ($P = 0.006$). Doxazosin (10^{-8} to 10^{-6} M) caused a significant concentration-dependent reduction of ^{3}H-5-HT binding to both the detrusor and urothelium (Table 1, Fig. 2). Non-specific binding established in the presence of excess unlabelled 5-HT was significantly lower (less than 5%) than the total binding (Fig. 2). Densitometric analysis of the data indicates that doxazosin is an effective inhibitor of 5-HT binding.

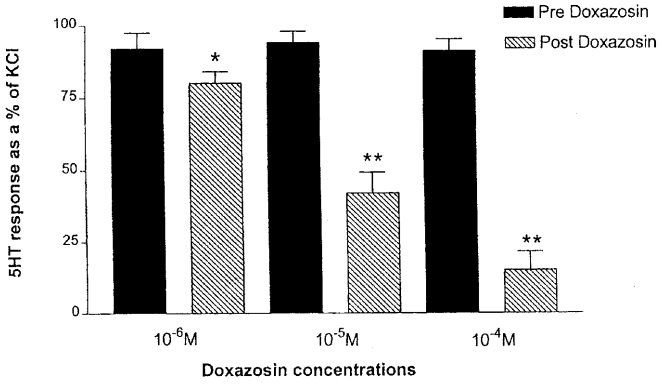

Fig. 1 The effect of doxazosin (10^{-6} to 10^{-4} M) on 5-hydroxytryptamine (*5-HT*)-mediated (10^{-3} M) rabbit detrusor contractions. $^*P = 0.02$; $^{**}P = 0.002$

The doxazosin levels achieved in human plasma are in the order of 10^{-8} M at the standard dosage of 2–4 mg/day used for the treatment of BPH [2]. This value is similar to the doxazosin concentration we used in the binding studies (10^{-8} to 10^{-6} M). However, these therapeutic levels are considerably lower than the concentration of doxazosin used in the functional studies (10^{-6} to 10^{-4} M). This apparent discrepancy must be balanced against the fact that the concentration of 5-HT required to induce detrusor contractions was very high (10^{-3} M). As a comparison, the circulating plasma levels of this bioamine are of the order of 10^{-8} to 10^{-9} M [7]. It is not unusual to require high levels of agonists in order to elicit responses in vitro.

Discussion

Since the prostate surrounds the urethra, the enlargement of this gland results in urinary symptoms and voiding dysfunction [23]. Lower urinary tract symptoms associated with BPH may be sub-divided into either voiding or irritative or both. Voiding symptoms include a weak stream flow, urinary hesitancy, incomplete bladder voiding and terminal dribbling [23]. Urinary frequency, nocturia and urgency are considered as irritative symptoms and are often associated with detrusor instability [23]. Detrusor instability commonly occurs in BPH [25]. Currently three α_1-adrenoceptor subtypes have been identified: α_{1A}, α_{1B} and α_{1D} [16]. In the prostate and bladder neck, the α_{1A} receptor subtype predominates [31, 46], whereas β-adrenoceptors predominate in the bladder body [18]. Hence, α_1-adrenoceptor blockade is thought to improve voiding rather than irritative symptoms [22]. However, it has been shown that, after 14 weeks of treatment, the mean reduction in voiding and irritative BPH symptom severity scores were 39%, 43% and 35% in doxazosin-treated (2 mg/day, 4 mg/day or 8 mg/day) patients compared with 17%, 20% and 15%, respectively, in placebo-treated patients [15]. These differences were significant ($P < 0.05$) [15]. Hence, doxazosin, a non-selective α_1-adrenoceptor antagonist, can improve both voiding and irritative symptoms associated with BPH [15].

Several 5-HT receptor subtypes mediate the numerous physiological actions of this bioamine [19, 40]. For example, 5-HT enhances vascular smooth muscle proliferation [32] and contractility [34]. In the urinary bladder, 5-HT has potent contractile activity in man [24] and numerous animal species, including the rabbit [6]. In addition, 5-HT can enhance inflammatory responses and the perception of pain [44].

We demonstrated that doxazosin can inhibit in vitro 5-HT-mediated detrusor contractions in a concentration-dependent manner. Autoradiography also demonstrated that doxazosin reduces 5-HT binding to its receptor sites, in a concentration-dependent manner, both in the detrusor and urothelium. Ondansetron, a 5-HT_3-receptor antagonist, also inhibited 5-HT-media-

Table 1 The effect of doxazosin (10^{-8} to 10^{-6} M) on [^{3}H]-5-hydroxytryptamine (*5-HT*) binding sites in the rabbit detrusor

	Control	Doxazosin (M)		
		10^{-8}	10^{-7}	10^{-6}
[3H]-5-HT binding (dpm × 1000/mm^2)				
Median	2.51	2.12*[1,*3]	1.80*[1,*4]	1.70*[2]
Range	2.34–3.06	1.97–2.66	1.58–2.38	1.38–1.83

*[1] $P = 0.002$ for control vs doxazosin (10^{-8} to 10^{-7} M); *[2] $P = 0.0005$ for control vs doxazosin (10^{-6} M); *[3] $P = 0.05$ for doxazosin 10^{-8} M vs doxazosin 10^{-7} M; *[4] $P = 0.01$ for doxazosin 10^{-7} M vs doxazosin 10^{-6} M

ted detrusor contractions in the presence of phenoxybenzamine. This demonstrates that phenoxybenzamine is unlikely to inhibit 5-HT_3 receptors, and that doxazosin inhibits 5-HT-mediated contractions by inhibiting 5-HT_3 receptors. Therefore, in addition to its established α_1-adrenoceptor-inhibiting activity, doxazosin also inhibits 5-HT-mediated action and binding in the rabbit. The ability to inhibit 5-HT-mediated detrusor contractions may play a role in the improvement of irritative symptoms but not necessarily detrusor instability. However, as increased bladder contractile response to 5-HT is thought to be associated with detrusor instability, as demonstrated in animal models [28, 35, 36, 37], a beneficial effect on detrusor instability cannot be ruled out. Phenoxybenzamine, as well as being an α-adrenoceptor antagonist, has been shown to have 5-HT_2-antagonist activity [17]. However, 5-HT induced detrusor contractions in the presence of phenoxybenzamine. Therefore, it is possible that phenoxybenzamine does not affect 5-HT_3 receptors that are present in the rabbit detrusor [3] or that the high concentration of 5-HT (10^{-3} M) used to induce contractions was able to overcome its antagonist activity.

The contractile effect of 5-HT on the urinary bladder is mediated by direct and indirect actions in man [8, 24] and various animal species, including the rabbit [6, 9, 39]. This bioamine may act directly by interacting with smooth muscle receptors or indirectly by stimulating the neuronal release of acetylcholine (ACh), indicating the involvement of the parasympathetic pathway. ACh subsequently acts on muscarinic receptors to produce detrusor contraction [10]. As far as we could establish

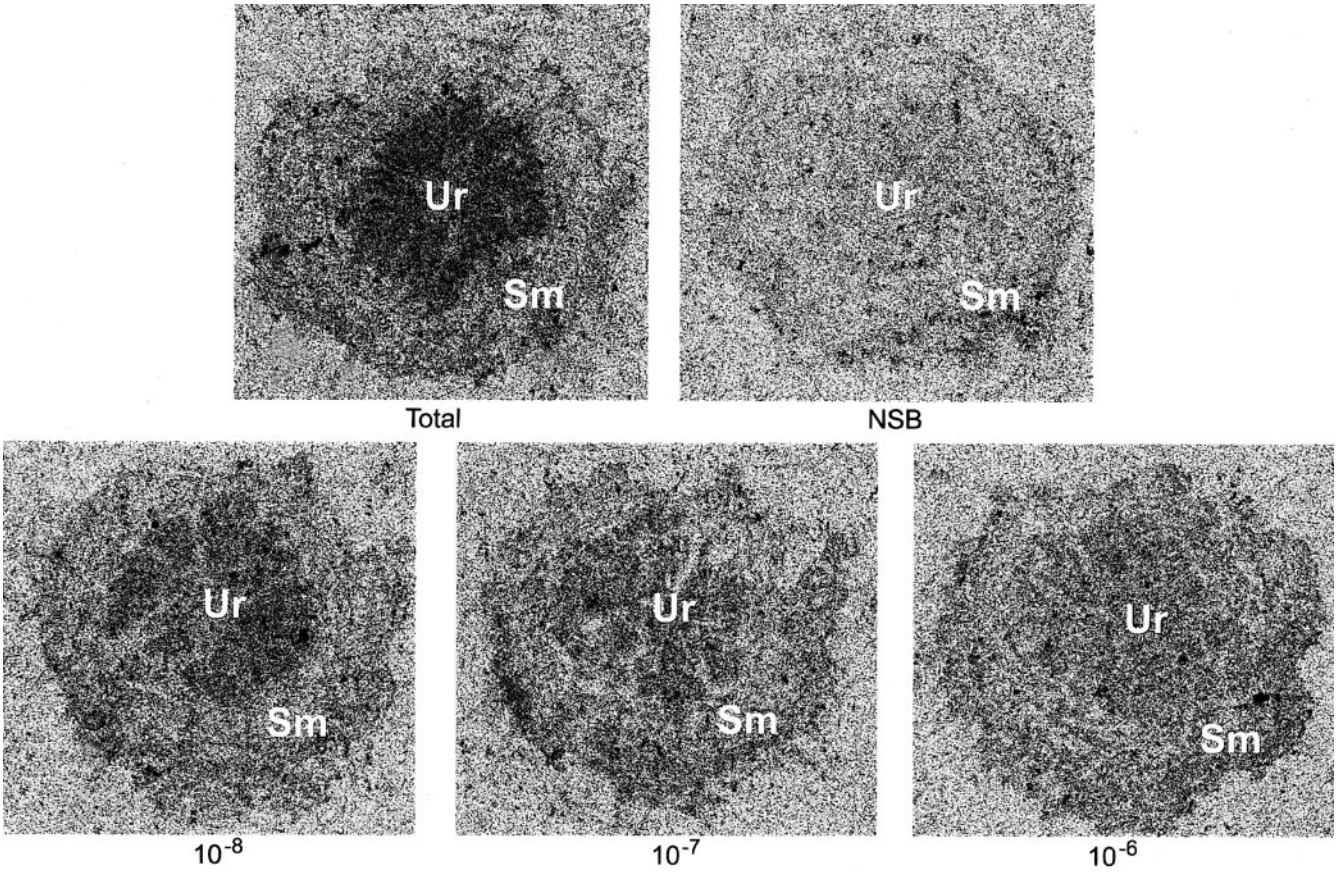

Fig. 2 *Top*: Low-resolution autoradiographs showing 5 nM [^{3}H]-5-HT binding to rabbit detrusor and urothelial sections (*Total*) and non-specific binding (*NSB*) determined in the presence of 10 μM 5-HT. *Bottom*: Concentration-dependent reduction in [^{3}H]-5-HT binding in the presence of doxazosin (10^{-8} to 10^{-6} M). (*Sm* Smooth muscle, *Ur* urothelium)

from the literature, there is no evidence that ACh-dependent nitric oxide release occurs in the urinary bladder.

In summary, we have demonstrated that doxazosin has 5-HT antagonist activity in the rabbit urinary bladder. This drug may also act as a 5-HT inhibitor at neuronal presynaptic receptor sites, thus preventing the release of ACh. Therefore, in addition to its α_1-adrenoceptor-inhibiting activity, doxazosin also inhibits 5-HT-mediated detrusor contractions. The 5-HT-inhibiting action of doxazosin appears to be non-selective, since doxazosin inhibits 5-HT_2 receptor-mediated platelet shape change [21] as well as 5-HT_3 receptor-mediated contractions (present study). Therefore, it is possible that doxazosin also has 5-HT_4 receptor inhibiting activity. This property may contribute to the observed improvement of irritative symptoms associated with BPH in patients treated with doxazosin. However, experimental work on the human bladder is needed to confirm that doxazosin does inhibit 5-HT_4 receptor-mediated contractions.

There remains the possibility that this compound also affects other receptor systems involved in detrusor dysfunction. This possibility warrants further investigation. It is not known whether other uro-selective α_1-adrenoceptor antagonists (e.g. tamsulosin, alfuzosin) have any 5-HT inhibiting activity or to what extent they improve irritative symptoms in patients with BPH. This information may help define the role of 5-HT in the pathogenesis of irritative symptoms in patients with BPH (or other bladder conditions).

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