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Up-regulation of endothelin (ET_A and ET_B) receptors and down-regulation of nitric oxide synthase in the detrusor of a rabbit model of partial bladder outlet obstruction

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Abstract Bladder outlet obstruction (BOO) is associated with altered bladder structure and function. Endothelin-1 (ET-1) has mitogenic and potent contractile properties. There are two ET receptors: ET_A and ET_B. Nitric oxide synthase (NOS) is the enzyme responsible for the synthesis of nitric oxide (NO) which is involved in smooth muscle relaxation. We investigated whether there are any changes in the density of ET-receptors and NOS in the detrusor and bladder neck in a rabbit model of BOO. Partial BOO was induced in adult male New Zealand White rabbits. Sham operated age-matched rabbits acted as controls. After six weeks the urinary bladders were excised and detrusor and bladder neck sections incubated with radioligands for ET-1, ET_A and ET_B receptors and with [^{3}H]–l-NOARG (a ligand for NOS). NADPH histochemistry was also performed. BOO bladder weights were significantly increased ($P = 0.002$). ET-1 binding and ET_A receptor binding sites were significantly increased in the BOO detrusor smooth muscle ($P = 0.04$, $P = 0.03$ respectively) and urothelium ($P = 0.002$, $P = 0.02$ respectively). ET_B receptor binding sites were also significantly increased in the BOO detrusor smooth muscle ($P = 0.04$). However, there was no change in the BOO bladder neck. NOS was significantly decreased in the detrusor smooth muscle ($P = 0.003$) and urothelium ($P = 0.0002$). In the bladder neck NOS was also significantly reduced in the urothelium ($P = 0.003$). NADPH staining was decreased in the detrusor and bladder neck. The up-regulation of ET receptors along with the down-regulation of NOS in the detrusor may contribute to the symptoms associated with BOO. Since ET-1 has a mitogenic role, especially via its ET_A receptors, the increase in ET_A receptors may also be involved in detrusor hyperplasia and hypertrophy in BOO. ET antagonists may therefore have a role in the treatment of patients with BOO.

Key words Endothelin receptors · Nitric oxide synthase · Rabbit · Bladder obstruction

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Introduction

Bladder outlet obstruction (BOO) results in significant changes in bladder structure and function [41]. These include detrusor hypertrophy/hyperplasia [7], elevated voiding pressures [37] and detrusor instability [38]. Detrusor instability describes a urodynamic phenomenon defined in 1988 as 'the unstable bladder'; in this state it is shown objectively to contract, spontaneously or on provocation, during the filling phase while the patient is attempting to inhibit micturition [14]. To further understand the pathophysiology of BOO, numerous animal models have been produced [10, 31, 26, 25, 11]. Despite this, our knowledge of the underlying pathophysiology of many of these changes, particularly detrusor instability, still remains uncertain.

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide [44]. Two major ET receptors have been identified and cloned: ET_A and ET_B [2]. In humans and rabbits, ET-1 is synthesised by vascular and nonvascular smooth muscle cells and by fibroblasts in the urinary bladder [32]. The extensive distribution of ET-1 synthesis in the urinary bladder, occurring in almost all cell types, suggests that this peptide could play a role in bladder wall modelling, the control of bladder smooth muscle tone and the regulation of local blood flow [32]. The activity of ET-1 is probably mediated via both autocrine [32] and paracrine [13] mechanisms. ET-1 elicits concentration-dependent contractions in smooth muscle strips from human and rabbit urinary bladders indicating the presence of functional ET receptors in both these species [32, 23]. ET_A receptors mediate the vasoconstrictor action of

ET-1 [20]. This response is related to the stimulation of phospholipase C activity that leads to the formation of inositol 1,4,5-trisphosphate and diacylglycerol. The former increases the intracellular calcium concentration, which in turn causes vasoconstriction [35].

The role of ET-1 is modulated by nitric oxide (NO) released by the endothelium [20]. NO is synthesised from-l-arginine by NO synthase (NOS) [5]. ET-1 and NO are widespread cell signalling molecules that have opposite effects. ET-1, the most potent endogenous vasoconstrictor and NO, the most potent endogenous vasodilator, form a paracrine/endocrine control cycle with a negative feedback mechanism [13]. Besides their contrasting and antagonistic actions, ET-1 and NO may regulate each other's synthesis [1]. ET-1 binding to its ET_B receptors is thought to mediate vasorelaxation by stimulating nitric oxide (NO) formation [12]. However, ET_B receptors may also mediate smooth muscle cell contraction [43].

Several techniques have been developed to study the distribution and activity of NOS, as an indirect measure of NO. This has previously been studied in the urinary tract using the NADPH-diaphorase technique [36] and anti-NOS antibodies [30]. These techniques allow the distribution of NOS to be determined with greater sensitivity, but cannot quantify the activity of the l-arginine:NO pathway. Previous autoradiographic studies have described the localisation of NOS binding sites in both brain [18] and peripheral tissues [39], using [^{3}H]–l-N^G-nitroarginine ([^{3}H]–l-NOARG) as a radioligand. This allows a quantitative assessment of receptor binding sites, an important factor when attempting to compare control and experimental tissue.

Diabetes mellitus (DM) is associated with atonic or hyper-reflexic bladders [17]. These abnormalities may relate to changes in ET receptor density and NOS binding sites as we have recently documented an increased expression of ET_B receptors and increased NOS binding sites in the detrusor and bladder neck of diabetic rabbits [26–28].

The rabbit model of partial BOO is thought to be representative of BPH in humans [3]. Hence, we used this model to investigate ET receptors and the density and distribution of NOS binding sites in the detrusor and bladder neck to determine whether there are any changes that may play a role in the pathophysiology of BOO.

Materials and methods

Animals

Adult male New Zealand White (NZW) rabbits (3 kg) were used ($n = 6$; each for sham operated controls and six-week partial BOO). All animals were fed ad libitum with SDS standard plain diet (SDS, Witham, UK) and allowed free access to water.

Induction of partial bladder outlet obstruction

Under general anaesthesia, a urinary catheter (Foley, C.R. Bard International Ltd, Crawley, UK) size 8 Fr gauge was inserted. After performing a lower midline laparotomy, a silk ligature was applied around the proximal urethra. The urinary catheter was then removed. The laparotomy incision was then closed and the animal allowed to recover. Sham operated controls underwent the same surgical procedure without inserting a proximal urethral ligature.

Post-operatively, the animals were given, subcutaneously, once daily 50 mg of enrofloxacin (Bayer, Newbury, UK) antibiotic for 5 days as a prophylaxis against infection.

Blood sampling

Blood was sampled at weekly intervals, via the middle ear vein, for serum creatinine, urea and electrolytes.

Preparation of tissues for autoradiographs

Following cervical dislocation, the urinary bladders from six-week partial BOO and age-matched sham operated controls were excised and weighed. They were subsequently divided into detrusor and bladder neck at the level of ureteric orifice. The tissues were then stored immediately at 70°C in airtight containers until used. The tissues stored at 70°C were subsequently mounted in AMES OCT embedding compound (BDH Laboratory Supplies, Poole, UK) and transverse sections (10 μmm) were cut in a cryostat at approximately 20°C and thaw-mounted onto gelatinised microscope slides. The slides were stored at 70°C in airtight containers until use.

Autoradiographic studies

Preliminary ET-1 binding studies (saturation analysis) were performed, where consecutive serial 10 μmm transverse sections were pre-incubated in 50 mM Tris HCl buffer, pH 7.4, for 15 min at 22°C in order to reduce endogenous peptide levels. These were then incubated (2 h at 22°C) in buffer containing 5 mM $MgCl_2$, 1% bovine serum albumin and 100 kiu/ml aprotonin in the presence of 0.003–1 nM ^{125}I-labelled ET-1 (Amersham International, Amersham, UK). The ^{125}I-labelled ET-1, ET_A and ET_B binding sites were identified using the selective radioligands ^{125}I-labelled PD151242 (ET_A) [4] and ^{125}I-labelled BQ3020 (ET_B) [33] (Amersham International, Amersham, UK). Non-specific binding was established by incubating adjacent sections in the presence of 1 μmmol/l unlabeled ET-1 (Bachem Fine Chemicals, Switzerland). Sections were then washed (twice for 10 min at 4°C) and binding determined by wiping sections from microscope slides and measuring ^{125}I bound in a gamma counter. Receptor affinity (K_D) was calculated using GraphPad Inplot Software (Graph Pad, San Diego, California, USA).

Quantitative assessment of ^{125}I-labelled ET-1, ^{125}I-labelled PD151242 (ET_A) and ^{125}I-labelled BQ3020 (ET_B) binding to rabbit urinary bladder

Serial consecutive 10 μmm sections from control and six-week partial BOO detrusor ($n = 18$ each for control and BOO; three sections were taken from each animal) and bladder neck ($n = 18$ as for detrusor) were pre-incubated, as described above.They were then incubated for 120 min, at 22°C, in buffer containing 0.15 nM ^{125}I-labelled ET-1, 0.15 nM, ^{125}I-labelled PD151242 and 0.3 nM ^{125}I-labelled BQ3020 (concentration above the K_D values determined from the saturation study). The degree of non-specific binding was established by incubating alternate detrusor ($n = 18$, as described above) and bladder neck sections ($n = 18$; as described above) in the presence of 1 μmM unlabelled ET-1. Slides were washed twice in buffer for 10 min, dipped in 4°C distilled water and then dried in a stream of cold air. Low-resolution autoradiography was carried out by exposing sections to Hyperfilm 3H (Amersham International, Amersham, UK) in X-ray cassettes for 3 days. Densitometric analysis was performed using an imaging system (Model GS-700 Imaging Densitometer, BIO-RAD, Hertfordshire, UK).

Binding was expressed in terms of radioligand bound (disintegrations per min; dpm) per unit area (mm^2), calculated from standard curves generated by ^{125}I microscales (Amersham International, Amersham, UK) that were co-exposed with tissue sections.

Microscopic localisation (high-resolution autoradiography) of binding sites was performed by post-fixing the tissues in paraformaldehyde vapour (2 h at 80°C) and coating the slides with molten nuclear emulsion (LM-1, Amersham International, Amersham, UK). Slides were then stored in light proof boxes with dessicant for up to 8 days at 4°C, after which they were processed in D19 high contrast developer (Kodak, UK) and fixed (Hypam, Ilford, UK). The underlying tissues were stained with haematoxylin and eosin (H&E) and high-resolution autoradiographs were viewed on an Olympus BX50 microscope and selected tissues photographed where appropriate.

Quantitative assessment of [^{3}H]–l-NOARG (NOS) binding to rabbit urinary bladder

This was carried out essentially the same way as for ET-1, ET_A and ET_B as described above. Consecutive serial sections from the control and six-week partial BOO detrusor ($n = 18$; as described above) and bladder neck ($n = 18$; as described above) were incubated in Tris buffer containing 3 mM $CaCl_2$ and 10nM [^{3}H]–l-NOARG (specific activity 55 Ci/mmol; Amersham International, Amersham, UK) for 60 min at 4°C. The degree of non-specific binding being established by incubating alternate detrusor ($n = 18$; as described above) and bladder neck sections ($n = 18$; as described above) sections in the presence of 10 μmM unlabelled l-arginine. After incubation, the slides were washed in buffer (four times for 2 min) to reduce non-specific binding, dipped in glass distilled water (4°C) and dried in a stream of cold air. Low-resolution autoradiography was carried out by exposing sections to Hyperfilm ^{3}H (Amersham International, Amersham, UK) in X-ray cassettes for 12 weeks. Densitometric analysis was performed as described above. The degree of binding was also expressed as described above.

NADPH diaphorase histochemistry

Localisation of putative NO-producing cells was performed by NADPH diaphorase histochemistry as recently described [30]. Briefly, control and six-week partial BOO detrusor ($n = 6$ each) and bladder neck ($n = 6$ each) sections, as used for autoradiographic analysis, were fixed for 30 min in 3% paraformaldehyde at 4°C. They were subsequently rinsed and incubated for 1 h at 37°C with 1 mg/ml βb-NADPH and 0.2 mg/ml nitro blue tetrazolium dissolved in 0.1 M phosphate buffer (pH 7.6) containing 0.2% Triton X-100. The sections were then rinsed under running tap water and stained with eosin. The NADPH diaphorase-reactive cells (blue staining) were observed by using an Olympus BX50 microscope (London, UK). In control experiments in which NADPH was excluded, no staining occurred. Selected sections were photographed.

Statistical analysis

All data are presented as median and range. Mann-Whitney two-tailed tests 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

Animal and bladder weights and serum creatinine, urea and electrolyte concentrations

The starting and final weight in both the control and six-week partial BOO rabbits were similar (data not shown). However, the six-week partial BOO rabbit urinary bladders were significantly heavier [2.1 g (1.8–2.3) vs 23 g (14–28); $P = 0.002$] than the age matched controls. There were no significant changes in the plasma creatinine, urea and electrolytes in either the control or six-partial BOO rabbits (data not shown).

Autoradiography

Binding studies confirmed that ^{125}I-labelled ET-1, ^{125}I-labelled PD151242 and ^{125}I-labelled BQ3020 all bound in a concentration-dependent manner to the detrusor and bladder neck sections. Saturation analysis showed that binding was to high affinity sites with K_D values in the sub-nanomolar range. Fixed concentrations of the radioligands (based on the K_D values) were then subsequently used in the autoradiographic experiments.

ET-1, ET_A, ET_B receptor and [^{3}H]–l-NOARG binding sites

There was dense ^{125}I-labelled ET-1, ^{125}I-labelled PD151242 (ET_A), ^{125}I-labelled BQ3020 (ET_B) and [^{3}H]–l-NOARG binding to all the detrusor (Fig. 1 and 2) and bladder neck (data not shown) sections in the control and six-week partial BOO rabbits. The non-specific binding in the presence of 1 μmM unlabelled ET-1 was less than 10% for ^{125}I-labelled ET-1, ^{125}I-labelled PD151242 and ^{125}I-labelled BQ3020 (Fig. 1). Similarly, the specificity of [^{3}H]–l-NOARG binding to tissue sections was confirmed by the significant reduction (>90%) in binding in the presence of l-arginine (Fig. 2).

Intense ^{125}I-labelled ET-1 binding was seen in the urothelium and smooth muscle cells of the detrusor and bladder neck in the controls and six-week partial BOO rabbits. Densitometric analysis of the film images indicated that ^{125}I-labelled ET-1 binding to the detrusor smooth muscle and urothelium significantly increased ($P = 0.04$, $P = 0.002$ respectively) in the six-week partial BOO rabbits compared to controls (Table 1 and Fig. 1).

The ^{125}I-labelled PD151242 and ^{125}I-labelled BQ3020 (ET_A and ET_B receptor binding sites, respectively) binding showed a similar distribution to ^{125}I-labelled ET-1 binding (Fig. 1). Densitometric analysis revealed a significant increase in ET_A ($P = 0.03$) and ET_B ($P = 0.04$) receptor binding sites in the six-week partial BOO detrusor smooth muscle (Table 1 and Fig. 1). ET_A receptor binding was also increased in the six-week partial BOO detrusor urothelium ($P = 0.02$) (Table 1 and Fig. 1). There was no change in the density of either ET_A or ET_B receptor binding in the bladder neck (data not shown).

NOS binding sites were significantly decreased in the six-week partial BOO detrusor smooth muscle ($P = 0.003$) and urothelium ($P = 0.0002$) (Table 2, Fig. 2). In the six-week partial BOO bladder neck NOS binding sites were also significantly decreased in the urothelium ($P = 0.003$) (Table 2, Fig. 3).

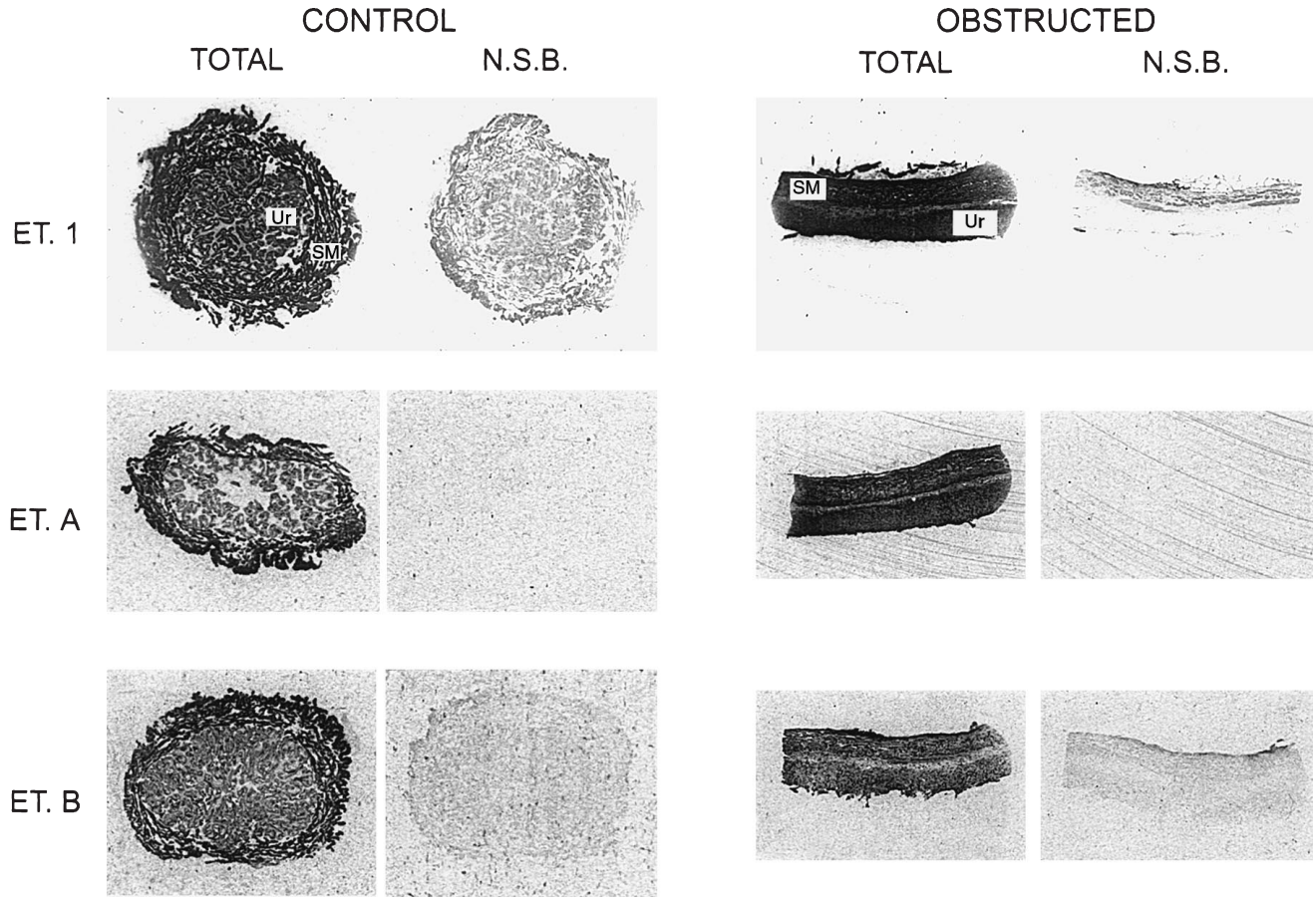

Fig. 1 ET receptor distribution on control and obstructed rabbit detrusor. Film autoradiographs (low-resolution) from control and obstructed sections incubated in ^{125}I-labelled ET-1 (ET.1), PD151242 (ET.A) and BQ3020 (ET.B) alone (total binding) and in the presence of excess unlabelled ET-1 (non-specific binding, N.S.B.). *Ur* urothelium, *SM* smooth muscle. Bar = 2.5 mm

Table 1 Photodensitometric analysis of ET-1, ET_A and ET_B receptor binding in the detrusor smooth muscle and urothelium of control and six-week partial bladder outlet obstruction (BOO) rabbits. Results are expressed as median and (range)

Receptor binding (dpm ×1000/mm^2)	Control median (range)	6-week BOO median (range)
Smooth muscle		
ET-1	61.7 (54.3–65.9)	69.7* (59.6–80.3)
ET_A	39.0 (23.7–49.5)	47.7 ** (39.0–63.6)
ET_B	29.6 (20.3–42.6)	33.7* (25.6–57.9)
Urothelium		
ET-1	38.7 (33.9–45.5)	57.8*** (49.6–67.4)
ET_A	23.0 (11.9–31.9)	38.4$^{\#}$ (27.6–49.7)
ET_B	21.9 (14.8–28.7)	25.2 (19.0–36.7)

Significant difference between corresponding medians *($P = 0.04$), **($P = 0.03$), ***($P = 0.002$), $^{\#}$($P = 0.02$)

Examination of the underlying tissues at high-resolution revealed marked binding of ET-1 to the urothelium, smooth muscle cells and blood vessels (Fig. 4). There was also marked binding of ET_A and ET_B receptors in the smooth muscle cells and blood vessels (Fig. 5 and 6 respectively). The [^{3}H]–l-NOARG binding sites were also similarly distributed (data not shown).

NADPH diaphorase histochemistry

Tissue sections from the control detrusor and bladder neck exhibited positive NADPH diaphorase activity (Fig. 7). The NADPH-diaphorase activity was evident predominantly in and around the urothelium of all regions and, to a much lesser extent, also in the smooth muscle. In the six-week partial BOO detrusor and bladder neck, NADPH-diaphorase activity was undetectable (Fig. 7).

Discussion

Benign prostatic hyperplasia (BPH) is the commonest known benign proliferative disorder. At the beginning of this decade almost 50% of all men aged 60–69 years in the UK had symptomatic BPH [6]. It affects more than 800,000 men in the United States each year [42]. An estimated 85% of all men older than 50 years have

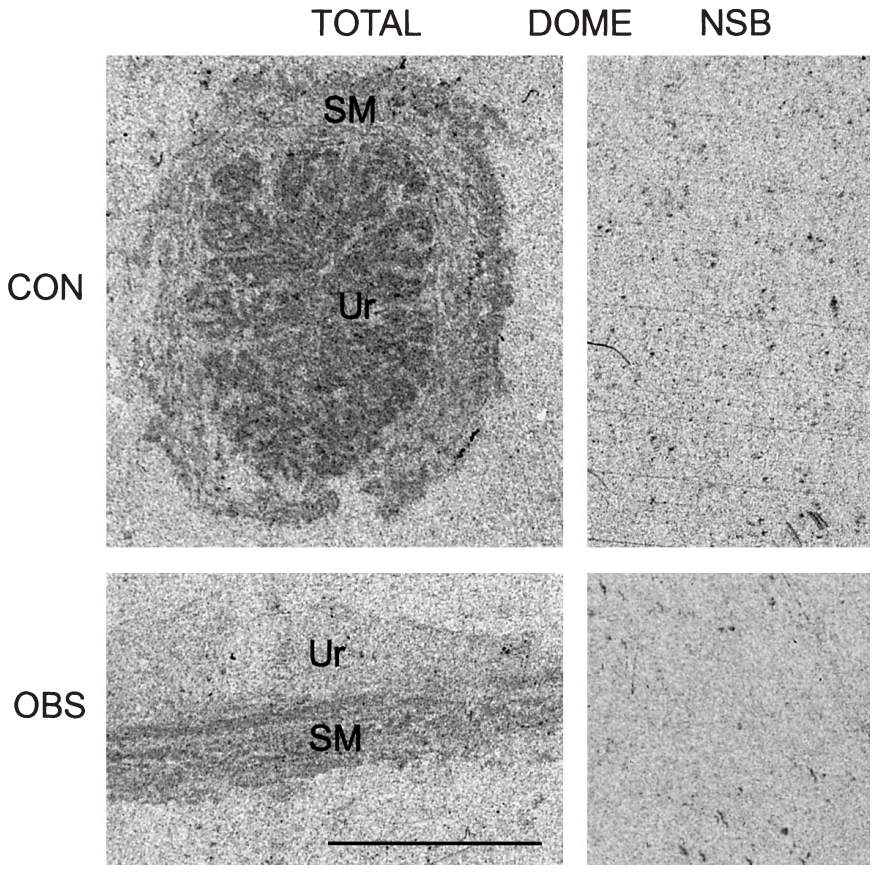

Fig. 2 [^{3}H]–l-NOARG binding to control and obstructed rabbit detrusor (*dome*). Film autoradiographs from sections of control (*CON*) and obstructed detrusor (*OBS*) incubated in [^{3}H]–l-NOARG alone (total binding) (identifying putative regions of NO synthase activity). Non-specific binding (*NSB*) determined in the presence of excess- l-arginine. *Ur* urothelium, *SM* smooth muscle. Bar = 2.5 mm

Table 2 Photodensitometric analysis of [^{3}H]–l-NOARG (NOS) binding sites in the detrusor and bladder neck smooth muscle and urothelium of control and six-week partial bladder outlet obstruction (BOO) rabbits. Results are expressed as median and (range)

Receptor binding (dpm × 1000/mm^2)	Control median (range)	6-week BOO median (range)
Detrusor		
Smooth muscle	2.47 (2.05–2.7)	2.02* (1.56–2.65)
Urothelium	2.84 (2.1–3.05)	1.53** (1.07–1.60)
Bladder neck		
Smooth muscle	2.57 (1.33–4.07)	2.38 (1.64–3.89)
Urothelium	2.4 (1.9–3.66)	1.83* (1.35–2.60)

Significant difference between corresponding medians *(P = 0.003), **(P = 0.0002)

symptoms arising from BPH [29]. By the ninth decade of life 50% of all American men require treatment for symptomatic relief of clinical BPH [29]. Symptoms of BPH are either obstructive or irritative or both. Obstructive symptoms are the result of urethral narrowing and include a weak stream flow, urinary hesitancy, incomplete bladder voiding and terminal dribbling [15]. Urinary frequency, nocturia and urgency are considered as irritative symptoms and are often associated with detrusor instability [15]. Detrusor instability has been demonstrated to occur in more than 60% of men with BOO due to BPH [19]. Similar results have also been demonstrated in animal models of BOO [37].

In this study, using a rabbit model of partial BOO, we have demonstrated that after six weeks there is a significant increase in the bladder weights. We also report a significant increase in ET-1, ET_A and ET_B receptor binding sites in the six-week partial BOO detrusor. High-resolution autoradiographs showed receptor binding to be associated with smooth muscle cells and blood vessels. NOS binding sites were also significantly decreased in the detrusor smooth muscle and urothelium and bladder neck urothelium of six-week partial BOO rabbits. NADPH-diaphorase activity was undetectable in the detrusor and bladder neck of six-week partial BOO rabbits compared to controls.

BOO has been shown to induce changes in the bladder morphology, physiology and pharmacology [21]. These changes include hypertrophy and hyperplasia of the bladder smooth muscle [7]. Similarly, one of the most striking features of experimentally-induced BOO is the increase in mass of this organ [24]. This change has been attributed to connective tissue deposition, tissue oedema and smooth muscle hypertrophy and/or hyperplasia [40].

ET-1 is known to induce smooth muscle cell mitogenesis [16]. This is thought to primarily involve ET_A receptors [16], although ET_B receptors have also been shown to

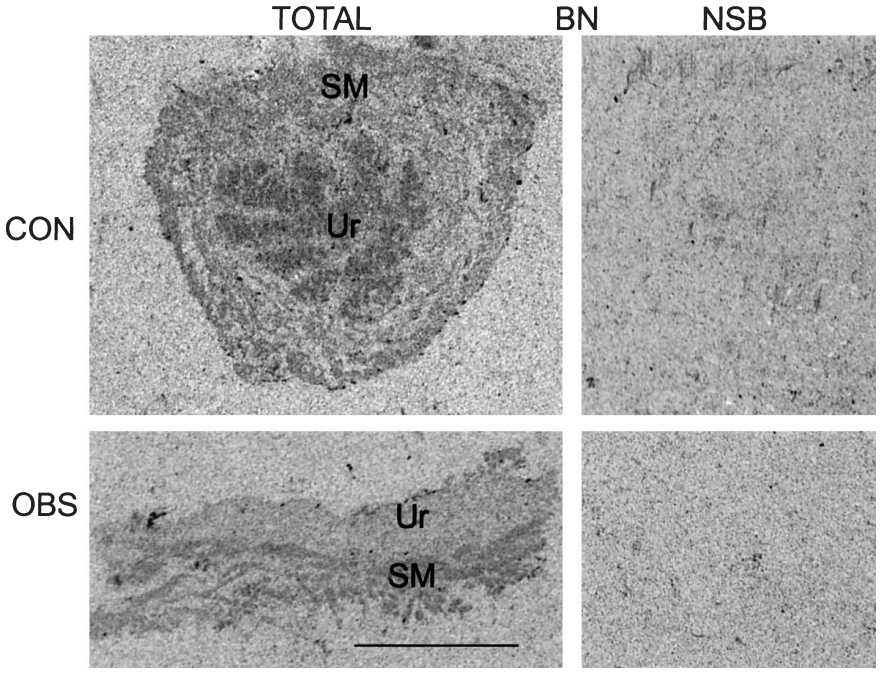

Fig. 3 [^{3}H]–l-NOARG binding to control and obstructed rabbit bladder neck (*BN*). Film autoradiographs of [^{3}H]–l-NOARG binding to control (*CON*) and obstructed (*OBS*) bladder neck, generated as in Fig. 2. Bar = 2.5 mm

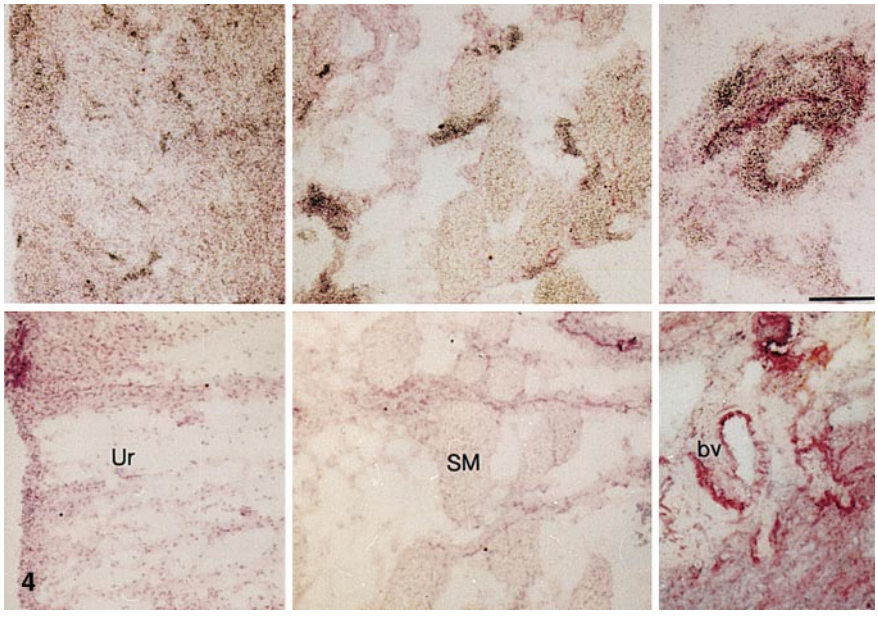

Fig. 4 High-resolution autoradiographs of [^{125}I]-ET-1 binding to obstructed detrusor. Autoradiographs generated on nuclear emulsion from a section of obstructed (6-week) detrusor incubated in [^{125}I]-ET-1. Binding evident as dark grains. Underlying tissue stained with H + E. *Top panels* Section incubated in [^{125}I]-ET-1 alone (total binding). *Lower panels* Section incubated in the presence of excess unlabelled ET-1 (non-specific binding). *Left* Urothelium (*Ur*), *Middle* smooth muscle (*SM*), *Right* Blood vessel (*bv*). Bar = 100 μm

play a role in cell proliferation [22]. Hence, the increase in ET_A and ET_B receptors along with increased ET-1 binding, in the rabbit model of partial BOO, may play a role in the increase in bladder weights through smooth muscle cell hyperplasia and hypertrophy. The increase in the bladder weights may be a compensatory response to the increased resistance to urinary flow produced in BOO. This change may enable the bladder to generate increased intravesical pressure to allow micturition to occur.

ET-1 is a potent vasoconstrictor peptide that is known to constrict detrusor smooth muscle [23]. ET_A receptors mediate the vasoconstrictor action of ET-1 by stimulating phospholipase C, which leads to the formation of inositol 1,4,5-triphosphate and diacylglycerol. The former increases the intracellular calcium concentrations, which in turn causes the vasoconstriction [35]. Currently, two ET_B-subtypes are thought to exist (ET_{B1} on endothelium cells mediating vasodilation via the NO system and ET_{B2}-mediating smooth muscle contraction) [43]. ET-1 is produced locally in the urinary bladder [32] and functions in a paracrine/autocrine manner [32, 13]. Hence, the up-regulation of ET_A and ET_B receptors and their subsequent stimulation by ET-1 may result in increased detrusor contractility.

NO synthesised from l-arginine by NOS [5] is a potent vasodilator. ET-1 and NO are thought to regulate each

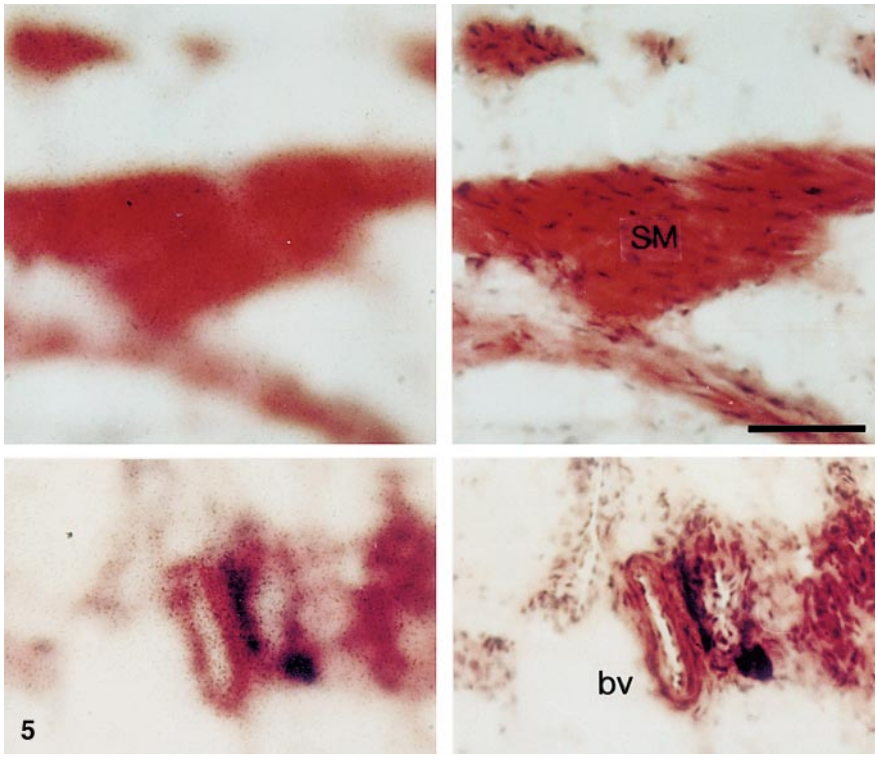

Fig. 5 High-resolution autoradiograph of ET_A binding to obstructed detrusor. Autoradiographs (generated on nuclear emulsion as in Fig. 4), from a section incubated in [^{125}I]-PD151242 (ET_A binding). *Left* Focussed on grains (ET_A binding sites). *Right* H + E-stained underlying tissue. *SM* Smooth muscle, *bv* blood vessel. Bar = 100 μm

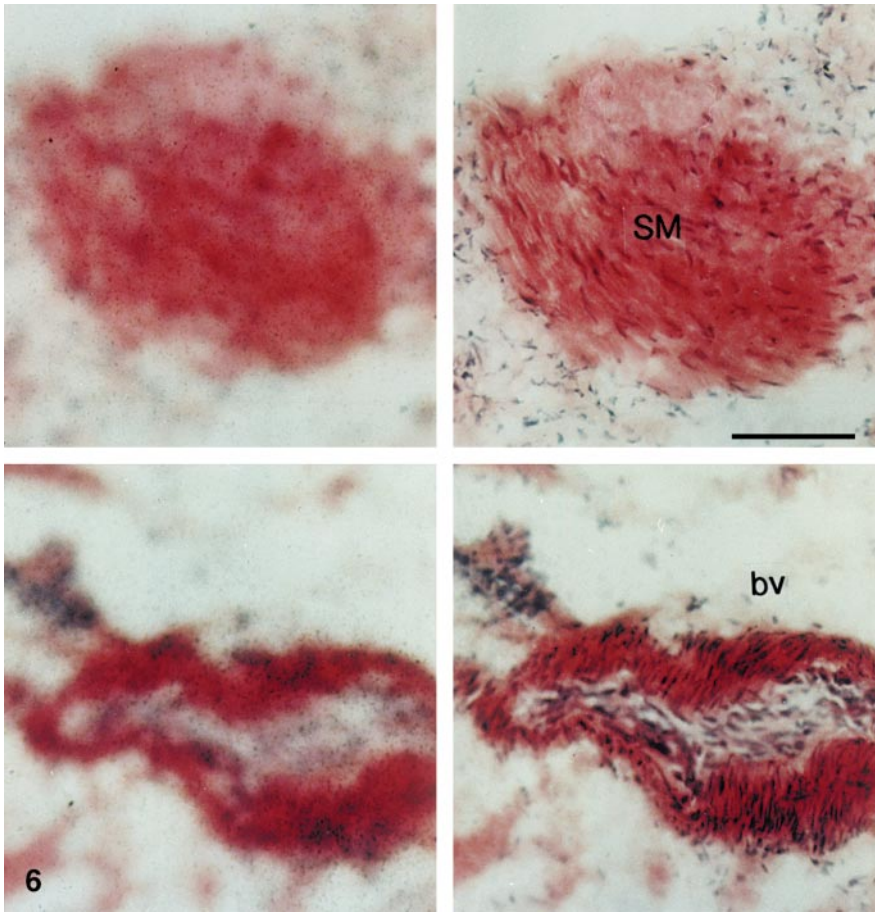

Fig. 6 High-resolution autoradiograph of ET_B binding to obstructed detrusor. Autoradiographs (generated on nuclear emulsion as in Fig. 4), from a section incubated in [^{125}I]-BQ3020 (ET_A binding). *Left* Focussed on grains (ET_B binding sites), *Right* H + E-stained underlying tissue. *SM* smooth muscle, *bv* blood vessel. Bar = 100 μm

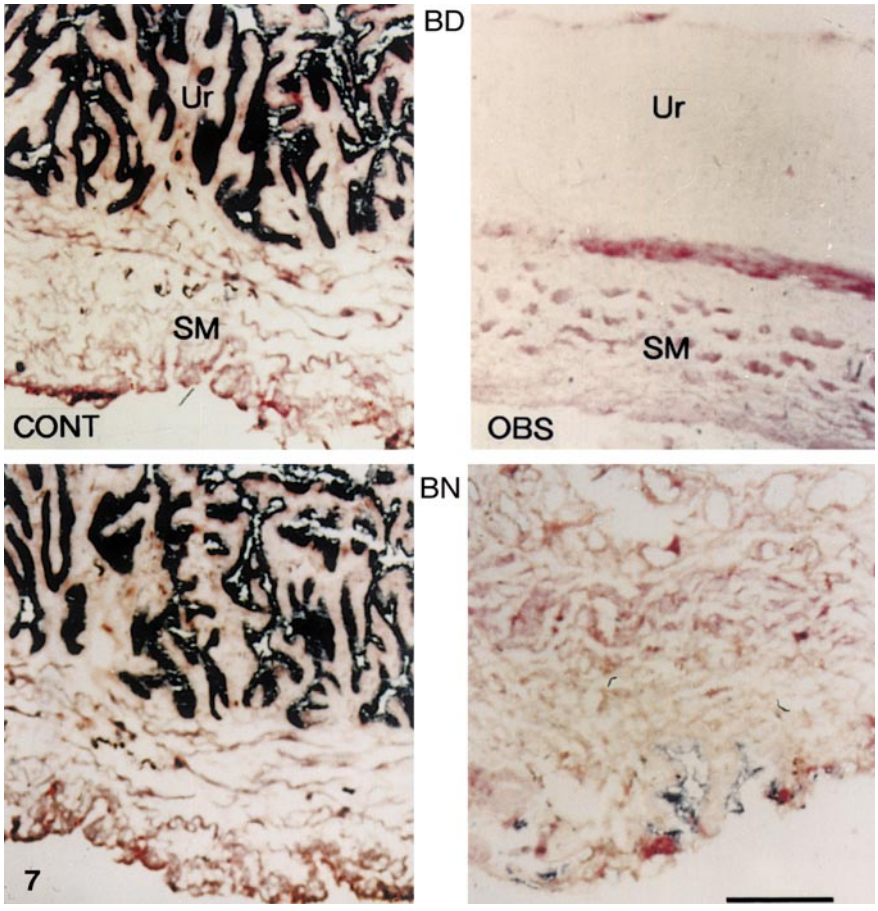

Fig. 7 NADPH histochemistry on control and obstructed rabbit detrusor and bladder neck. Photomicrographs from sections of control (*CONT*) and obstructed (6-week, *OBS*) detrusor (*BD*) and bladder neck (*BN*) used for NADPH histochemistry. Blue reaction product indicates regions of putative NO synthase activity. *Ur* urothelium, *SM* smooth muscle. Bar = 50 μm

other's synthesis. ET-1 acts upon ET_{B1} receptors and subsequently leads to the activation of the endothelial constitutive isoform of NOS and stimulates NO production [12]. However, NO can inhibit ET-1 synthesis [8]. The presence of NOS binding sites and NADPH-diaphorase activity in the control detrusor and bladder neck indicates that under normal conditions NO may have a physiological role (i.e. smooth muscle relaxation) in the urinary bladder. It may also be involved in the regulation of ET-1 synthesis and activity in the urinary bladder. However, in the six-week partial BOO rabbits, there is reduced urinary bladder NO synthesis indicated by a down-regulation of NOS binding sites and NADPH-diaphorase activity in the detrusor and bladder neck. Since NO inhibits ET-1, a decrease in the availability of NO in the urinary bladder may be associated with increased ET-1 production and may account for the increased ET-1 binding demonstrated in the six-week partial BOO detrusor. This, in turn, may lead to increased ET_A and ET_B receptor stimulation. Hence, the up-regulation of ET_A and ET_B receptors along with a down-regulation of NOS binding sites and NADPH-diaphorase activity may lead to increased detrusor contractility associated with partial BOO. These changes may initially be beneficial because it may enable the generation of increased intravesical pressure, during micturition. This will be needed to overcome increased resistance to urinary flow and may reflect any symptomatic improvement when BPH is managed by 'watchful waiting' [9]. However, detrusor instability can occur in the majority of men with BOO due to BPH [19] and similar results have also been demonstrated in animal models of BOO [37]. At present, it is thought that post-junctional denervation supersensitivity plays a role in obstructive detrusor instability [34], but little is known about how exactly it develops. Hence, with time, the up-regulation of ET receptors and down-regulation of NOS binding sites resulting in increased bladder contractility may be associated with raised intravesical pressure. Ischaemic changes in the vesical wall are thought to result from increased intravesical pressure. This in turn results in axonal degeneration associated with detrusor instability [45]. Therefore, the up-regulation of ET_A and ET_B receptors along with a decreased availability of NO may contribute to the pathophysiology of detrusor instability associated with obstruction. ET antagonists may, therefore, in the future, prove useful in the treatment of BOO, although more work is required to confirm this.

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