A Novel In-Vitro System for the Simultaneous Exposure of Bladder Smooth Muscle Cells to Mechanical Strain and Sustained Hydrostatic Pressure

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The novel hydrostrain system was designed in an effort to establish and maintain conditions that simulate the in-vivo mechanical environment of the bladder. In this laboratory system, ovine bladder smooth muscle cells on flexible, 10-cm-dia silastic membranes were exposed simultaneously to hydrostatic pressure (40 cm H_2O, a pressure level currently associated with bladder pathologies) and mechanical strains (up to 25 percent) under standard cell culture conditions for 7 h. Under these conditions, Heparin Binding-Epidermal Growth Factor and Collagen Type III mRNA expression were significantly increased ($p<0.01$ and 0.1, respectively); however, no changes were observed in Collagen Type I mRNA expression. Decreases in the Collagen Type I:Type III ratio following simultaneous exposure of bladder smooth muscle cells to pathological levels of hydrostatic pressure and mechanical strain in vitro are in agreement with clinically observed increases in Collagen Type III with concomitant decreased human bladder compliance. The results of the present study, therefore, provide cellular/molecular level information relevant to bladder pathology that could have significant implications in the field of clinical urology. [DOI: 10.1115/1.1449903]

Keywords: Hydrostrain System; Bladder; Smooth Muscle Cells; Mechanical Forces; mRNA; Collagen Type I; Collagen Type III; Heparin Binding-Epidermal Growth Factor

Introduction

In vivo, the effects of mechanical forces on the bladder wall and its constitutive components are necessary for normal, physiological development and are integral to the function of this organ. For example, it has been hypothesized that poor development of the bladder wall in cases of exstrophy-epispadias complex and of bilateral single system ectopic ureters in females is due to lack of mechanical stimuli on the detrusor during development [1–3]. In contrast, conditions involving excessive mechanical force, such as bladder outlet obstruction, have been associated with hypertrophy of the bladder wall tissue, increased synthesis of extracellular matrix components, and subsequent loss of bladder tissue compliance [4–7]. When bladder outlet obstruction persists over many years, the cellular and mechanical consequences of increased bladder pressure and wall hypertrophy contribute not only to clinical deterioration of the bladder but, ultimately, to kidney damage and/or failure as well [8].

Several researchers [9–13] have exposed cultured bladder smooth muscle cells, the predominant cell line of the bladder wall tissue, to various types of mechanical forces in vitro. Results of such studies have provided evidence that exposure of smooth muscle cells to mechanical strain (up to 25 percent at a frequency of 1 Hz) leads to a time-dependent increase in Heparin Binding-Epidermal Growth Factor mRNA-levels (with maximum levels after 4 hours of exposure to strain) [9], increased HB-EGF promoter activity [9], and increased collagen Type III synthesis [10,11]. Subsequent work in our laboratories investigated the responses of cultured bladder smooth muscle cells to hydrostatic pressure and reported similar effects [12], as well as significant alterations in the control mechanisms for collagen turnover [13]. Although these systems have provided a starting point for understanding the cellular- and molecular-level responses of the bladder tissue to mechanical forces, they suffer from important limitations. For example, the fashion in which the mechanical stimulus was applied (i.e., multiple cycles of stretch and relaxation per minute [9–13] or isolated exposure to hydrostatic pressure in the absence of stretch [12]) failed, in many respects, to mimic the events occurring in the bladder under normal, physiologic conditions. In addition, the effects of hydrostatic pressure and of mechanical strain applied simultaneously (that is, in a manner which simulates the in-vivo mechanical environment of this organ) on bladder tissue function, as well as the mechanism(s) behind these responses, have not been investigated at the cellular/molecular level.

In order to address this issue, the present study involved the design of a novel laboratory system which would more closely simulate the in-vivo environment of the bladder wall tissue; such an in-vitro system must expose bladder cells simultaneously to both hydrostatic pressure and mechanical strain. For this reason, the novel hydrostrain system was designed to incorporate specifications pertinent to the in-vivo physiological and pathological mechanical environment of bladder tissue and was used in the present study to simultaneously expose bladder smooth muscle cells to hydrostatic pressure of 40 cm H_2O (a pressure level currently associated with bladder disease and kidney failure) and mechanical strains in the range of 5 to 25 percent. Strain variations of this magnitude are appropriate for the present model because bladder tissue is exposed to various regional mechanical/material

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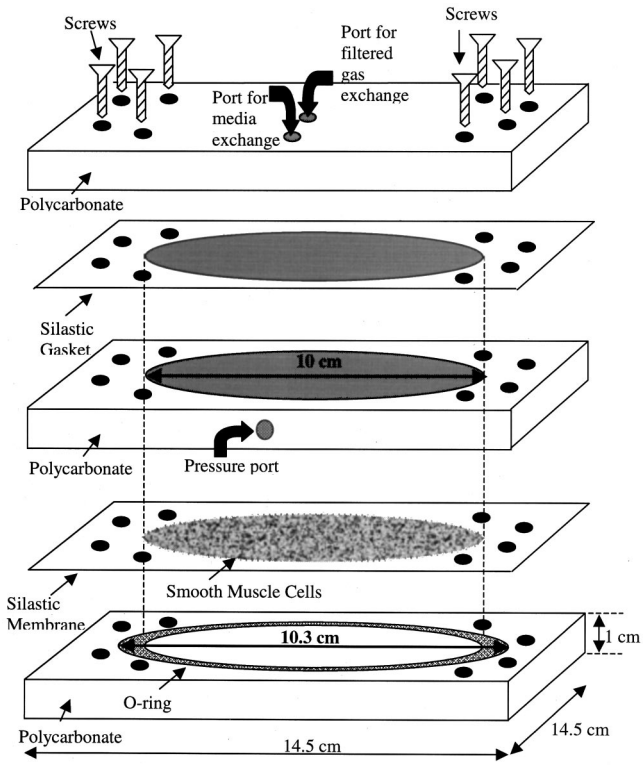

Fig. 1 Schematic diagram of the hydrostrain system. A schematic diagram (not to scale) of the Hydrostrain System used to expose bladder smooth muscle cells simultaneously to mechanical strains (up to 25 percent) and hydrostatic pressure (40 cm H_2O)

conditions in vivo; recent studies used computed tomography (CT) reconstructions and confirmed that extremely complex, and nonuniform, strain fields exist along various regions of the bladder wall tissue [14].

This novel, custom-designed, and fabricated laboratory system, along with in-vitro cellular models and molecular-level techniques, were then utilized in the present study to investigate the cellular and molecular-level responses of bladder smooth muscle cells simultaneously exposed to sustained hydrostatic pressure and to mechanical strain.

Materials and Methods

The Hydrostrain System

Preparation of Flexible Silastic Substrates. SYLGARD 184 Silicone Elastomer® (Dow Corning) was mixed (10:1, w/w) with a silicone resin curing agent (Dow Corning) and de-aired by exposure to vacuum at 25 in Hg for 30 min. The elastomer was then poured to a sheet (0.1 ± 0.003 cm thick) and cured at 23°C for 7 days. Following full cure, the silicone sheets were soaked in 1N HCl for 2 h, soaked in 1N NaOH for 1 hour, and sterilized in a steam autoclave at 250°F for 20 min.

Design of the Hydrostrain System. A novel laboratory setup that allows simultaneous exposure of bladder smooth muscle cells to mechanical strain and hydrostatic pressure was designed, assembled, and calibrated. This system (schematically shown in Fig. 1) consisted of a machine-milled polycarbonate stretching system, a 0.5-mm-thick silastic gasket (Dow Corning), a flexible, silastic membrane (1 mm thick; 10 cm in diameter; elastic modulus=20 MPa), and an O-ring. Filtered gas exchange was maintained through a Teflon syringe filter (0.2 μm pore diameter; Micron Separation Inc.), which was also attached to the top of the chamber. The hydrostrain system was fully assembled, screw-tightened, sterilized by autoclaving at 250°C for 20 min, and allowed to air dry at room temperature in a sterile environment overnight.

Strain Quantification. Radial strain quantification measurements (for a given applied pressure) were performed empirically by visualizing the circular flexible membrane deflection using video analysis [15]. Briefly, toner powder was used to imprint marks of concentric circles on the silastic membrane surface. The hydrostrain system was then placed under a microscope/video system (Nikon/Hitachi), where images of the undeformed and deformed (with deflection resulting from exposure to 40 cm H_2O pressure) circles were captured using Image Pro Plus (Media Cybernetics Image Analysis); images of undeflected and deflected silastic membranes were stored electronically for later analysis. A wax mold of the deflected membrane was also cast and used to determine the angle of inclination (with respect to a horizontal line of sight) of the deformed silastic membrane.

The average radial strain, ε_r, at a given location, r, for a specific applied pressure was determined by the following equation [15]:

$$\varepsilon_r = \frac{\frac{L_D}{\cos\theta} - L_O}{L_O}$$

where ε_r is the mechanical strain (a function only of radial position, r), L_D is the deformed thickness (in mm) of an arc of the membrane, L_O is the original thickness (in mm) of an arc of the membrane, and θ is the angle (in deg) of inclination.

Simultaneous Exposure of Bladder Smooth Muscle Cells to Mechanical Strain and Sustained Hydrostatic Pressure

Bladder Smooth Muscle Cell Isolation and Culture. Bladder smooth muscle cells were isolated from explants of neonatal lamb bladder muscularis using techniques we have previously described [16], were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco), supplemented with 10 percent fetal bovine serum (Gibco), and were cultured under standard cell culture conditions (a sterile, humidified, 37°C, 95 percent air/5 percent CO_2 environment). Smooth muscle cells were passaged using trypsin in a PET solution (an enzyme solution containing 0.01 percent ethylene glycol bis-(β-aminoethyl ether) N,N,N′,N′,-tetraacetic acid, 0.05 percent trypsin, 0.5 percent polyvinylpyrrolidine, 0.5 percent 4(-2-hydroxyethyl)-1-1 piperazineethanesulfonic acid, and 0.9 percent sodium chloride in distilled water; all chemicals were obtained from Sigma). The smooth muscle cell phenotype was confirmed using immunohistochemical staining for α-smooth muscle actin using the Multispecies Ultrastreptavidin Detection System (Signet Laboratories).

All experiments were performed using cells of passage 2–5.

Experiments. Prior to ovine bladder smooth muscle cell seeding (12,732 cells/cm^2), the silastic membranes were coated with sterile fibronectin (20 μg/mL; Sigma) overnight and then gently rinsed with sterile PBS. These cells were then cultured under standard cell culture conditions for one day. At that time, bladder smooth muscle cells were simultaneously exposed to mechanical strains of up to 25 percent and hydrostatic pressures of 40 cm H_2O for 7 consecutive hours. Designated hydrostatic pressures (and resulting strains) on the cell layer were achieved by applying (over approximately 30 min) and maintaining pressure with a 60-mL sterile syringe (Corning) attached to the pressure port (by a four-way stopcock).

Smooth muscle cells maintained under 0.3 cm H_2O pressure and no mechanical strain, on either flexible silastic substrates (10 cm dia) or rigid tissue-culture plastic-ware (10-cm-dia petri dishes), under similar cell culture conditions and time periods, were controls.

mRNA Expression of Heparin Binding-Epidermal Growth Factor (HB-EGF), Collagen Type I, and Collagen Type III. At the end of the experiments, mRNA from ovine bladder smooth muscle cells simultaneously exposed to pressure (40 cm H_2O) and mechanical strain (up to 25 percent) for 7 h, as well as from smooth muscle cells maintained under 0.3 cm H_2O pressure and no mechanical strain, but otherwise similar conditions (controls), were digested using Triazol (Gibco), extracted using chloroform, and precipitated using isopropanol.

Reverse transcriptase-polymerase chain reaction was performed using sample aliquots of 0.5–1.0 μg/mL RNA, and the Ambio Retroscript Kit with primers (200 pmole/μL; Gibco) specific to either heparin binding-epidermal growth factor (HHBEGF), collagen I (HSCOL1A2), or collagen III (HSCOL3A1). cDNA for heparin binding-epidermal growth factor (HB-EGF), Collagen I (Col I), Collagen III (Col III), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were then amplified through 25 cycles of denaturing (at 94°C for 3 min), denaturing (at 94°C for 1 min), annealing (at 60°C for 2 min), extending (at 72°C for 2 min), and finally extending (at 72°C for 7 min).

Electrophoresis of cDNA samples was performed in a Joey Gel Casting System (Owl) containing TBE running buffer across a constant voltage of 200 V for approximately 1.5 h. The amount of cDNA per lane (bands of either HB-EGF, Collagen I, Collagen III, or GAPDH) was then measured using a phospho-imager (Molecular Dynamics) and used as an indicator of mRNA expression.

All experiments were repeated three separate times.

Statistical Analysis. All numerical data were analyzed using Student's t-test.

Results

Radial Strain of Flexible, Silastic Membranes. The strain distribution on the circular, silastic membranes was nonuniform, biaxial, and composed of radial strains (ε_r) and circumferential strains (ε_θ). In this analysis, radial strains (which were dependent only upon radial location) were used as an indicator of strain magnitude. Table 1 summarizes the calculated average radial strain at various points along a representative circular, silastic membrane similar to those used in the present study following exposure to sustained hydrostatic pressures of 40 cm H_2O.

Table 1 Correlation between radial position (cm) and strain (percent) for flexible silastic membranes simultaneously exposed to sustained hydrostatic pressure and mechanical strain using the hydrostrain system

Radial Position (cm)	Strain (%)
1.50	19.8 ± 3.72
2.40	16.8 ± 6.59
3.50	9.66 ± 2.89
4.80	8.23 ± 4.38
6.30	4.91 ± 2.15
7.50	11.5 ± 4.64
8.90	21.4 ± 5.42

Effects of Mechanical Strain and Sustained Hydrostatic Pressure on Ovine Bladder Smooth Muscle Cell Function. Due to the hydrophobic nature of silastic, all such membranes were pre-coated with fibronectin (25 μg/mL) before use in cell experiments. Figure 2 illustrates bladder smooth muscle cells adherent onto either tissue culture polystyrene (Frame A) or onto these flexible, fibronectin-coated, silastic membranes (Frame B) under control conditions (that is, cells maintained under 0.3 cm H_2O pressure but no mechanical strain) for 7 h. These results provided evidence that the bladder smooth muscle cells used in the present study did adhere to the fibronectin-coated, silastic membranes; moreover, these cells expressed similar cell morphology both on the flexible, silastic membranes and on the rigid, tissue-culture polystyrene.

Using the hydrostrain system with the flexible, silastic membranes, ovine bladder smooth muscle cells were simultaneously exposed to mechanical strain of up to 25 percent and to hydrostatic pressure of 40 cm H_2O under standard cell culture conditions for 7 consecutive hours. mRNA levels for Heparin Binding-Epidermal Growth Factor (HB-EGF), Collagen Type I, and Collagen Type III were measured and normalized to references (that is, cells maintained on rigid, tissue-culture polystyrene substrates under 0.3 cm H_2O pressure but no mechanical strain; Figs. 3, 4, and 5, bar A).

Compared to control cells maintained on flexible, silastic substrates under 0.3 cm H_2O pressure but no mechanical strain (Fig. 3, bar B), HB-EGF mRNA levels of ovine bladder smooth muscle cells (in serum-free DMEM) exposed simultaneously to mechanical strains of up to 25 percent and to hydrostatic pressure of 40 cm H_2O (Fig. 3, bar C) increased by more than 25 percent; this increase was significant ($p<0.01$).

Collagen Type I mRNA levels of ovine bladder smooth muscle cells (in serum-free DMEM) simultaneously exposed to mechanical strains of up to 25 percent and to hydrostatic pressure of 40 cm H_2O (Fig. 4, bar C) were similar to levels obtained from control cells maintained on flexible, silastic substrates under 0.3 cm H_2O pressure but no mechanical strain (Fig. 4, bar B).

However, Collagen Type III mRNA levels of ovine bladder smooth muscle cells (in serum-free DMEM) on flexible, silastic membranes simultaneously exposed to mechanical strains of up to 25 percent and to hydrostatic pressure of 40 cm H_2O (Fig. 5, bar C) increased by 13 percent compared to the levels obtained from control cells maintained on flexible, silastic substrates under 0.3 cm H_2O pressure but no mechanical strain (Fig. 5, bar B).

Discussion

Despite the fact that bladder development and function are influenced by the mechanical forces that act on the bladder wall under both physiological and pathological conditions, the identity and magnitude of the mechanical stimuli which are responsible for maintaining bladder tissue integrity and/or affecting pathological change remain ill-defined. In order to understand the relationship between mechanical stimuli and bladder tissue function at the cellular level, some researchers have investigated the effects of axial stretch on bladder cell function [9–11] and have shown that exposure of bladder smooth muscle cells to mechanical strains (of up to 25 percent at a frequency of 1 Hz) leads to a time-dependent increase in Heparin Binding-Epidermal Growth Factor mRNA levels (with maximum levels after 4 h of exposure to strain) [9], increased HB-EGF promoter activity [9], and increased collagen Type III synthesis [10,11]. Although these studies [9–11] have provided some insight into the molecular-level changes in bladder cells following exposure to mechanical strain, the data are difficult to interpret clinically since the stimulus was applied in a manner that did not simulate the mechanical environment of the bladder wall.

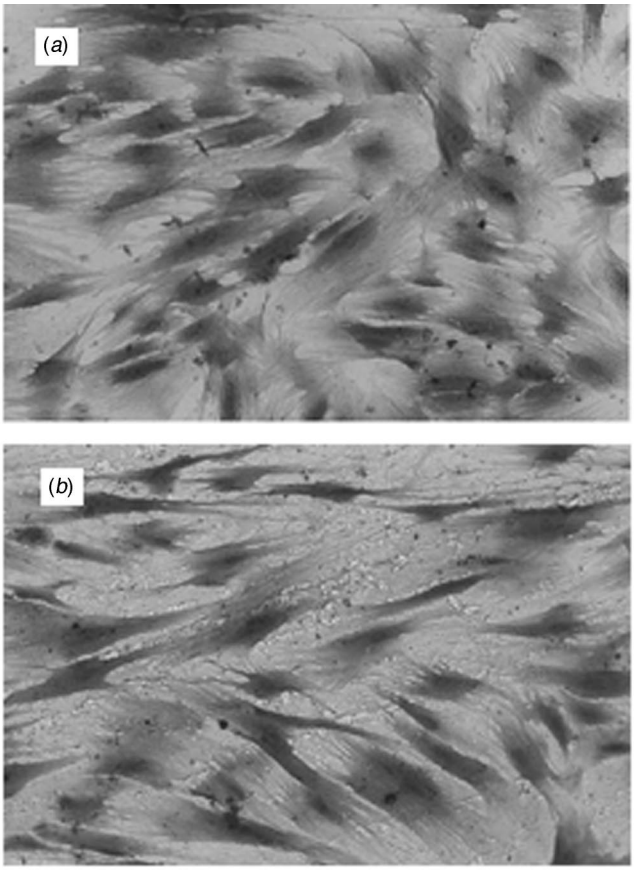

Fig. 2 Ovine bladder smooth muscle cells adherent onto the fibronectin-coated, flexible silastic membranes. Representative light micrographs illustrating bladder smooth muscle cells adherent onto either tissue culture polystyrene (Frame A) or onto the flexible, fibronectin-coated, silastic membranes (Frame B) under control conditions (that is, cells maintained under 0.3 cm H_2O pressure but no mechanical strain under standard cell culture conditions) for 7 hours. These results provided evidence that the bladder smooth muscle cells used in the present study did adhere to the fibronectin-coated membranes; moreover, these cells expressed similar cell morphology on the flexible, silastic membranes and on the rigid, tissue-culture polystyrene. Stain: Coomassie Blue. Magnification: 25X.

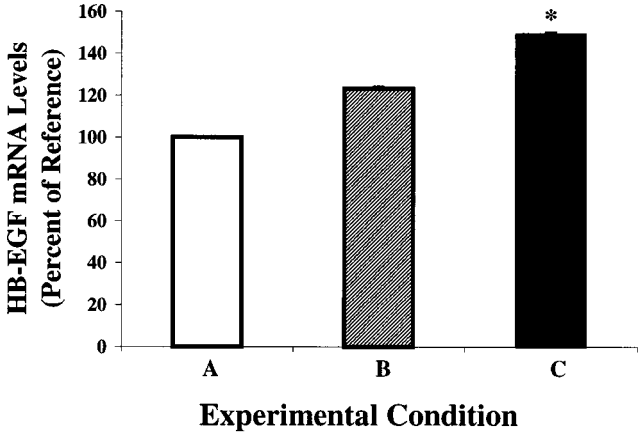

Fig. 3 Normalized HB-EGF mRNA expression by ovine bladder smooth muscle cells simultaneously exposed to mechanical strain of up to 25 percent and to hydrostatic pressure of 40 cm H_2O. HB-EGF mRNA levels of ovine bladder smooth muscle cells (in serum-free DMEM) on flexible, fibronectin-coated, silastic membranes simultaneously exposed to mechanical strain of up to 25 percent and to hydrostatic pressure of 40 cm H_2O (bar C) under standard cell culture conditions increased (by more than 25 percent) compared to control cells maintained on flexible, silastic substrates under 0.3 cm H_2O pressure but no mechanical strain (bar B). Data are mean values ±SEM of three experiments and were normalized with reference (that is, cells maintained on rigid, tissue-culture polystyrene substrates under 0.3 cm H_2O pressure but no mechanical strain; bar A) values taken as 100 percent. *$p<0.01$ compared to control cells maintained on flexible, silastic substrates under 0.3 cm H_2O pressure but no mechanical strain (bar B).

Previous work in our laboratories [12,13,16] investigated the responses of bladder smooth muscle cells (the predominant cell line of the bladder wall tissue) to hydrostatic (2–40 cm H_2O) pressure. Sustained hydrostatic pressure is an appropriate mechanical force to use when examining bladder smooth muscle cell function in vitro, because it provides a reasonable first approximation of the conditions under which the soft tissue of the bladder operates during the slow process of the filling cycle; in addition, hydrostatic pressure (in cm H_2O) is the parameter currently measured in clinical urodynamic evaluations in order to assess the adequacy of the human bladder for storing urine. In our studies involving hydrostatic pressure alone, cultured bladder smooth muscle cells exposed to various levels (4–40 cm H_2O) of sustained hydrostatic pressure exhibited significant changes in cell proliferation [16], a time-dependent upregulation in HB-EGF mRNA levels (with maximum values reaching three times that of controls levels) [12], increased HB-EGF promoter activity [12], and reduced metalloprotease activity favoring collagen accumulation [13].

Although the sustained pressure studies provided new information regarding mechanism(s) of select functions of bladder smooth muscle cells to physiological (0.3–10 cm H_2O) and to pathological (20 and 40 cm H_2O) levels of hydrostatic pressure, this work did not consider the second mechanical force which is known to be present within the bladder. Specifically, an in-vitro system that would more closely simulate the in vivo environment of the blad-

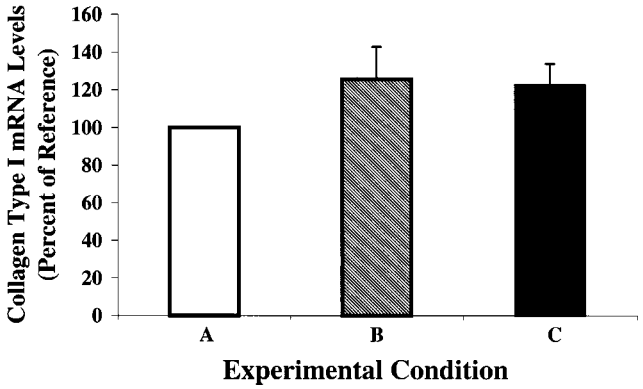

Fig. 4 Normalized collagen type I mRNA expression by ovine bladder smooth muscle cells simultaneously exposed to mechanical strain of up to 25 percent and to hydrostatic pressure of 40 cm H_2O. Collagen Type I mRNA levels of ovine bladder smooth muscle cells (in serum-free DMEM) on flexible, fibronectin-coated, silastic membranes simultaneously exposed to mechanical strain of up to 25 percent and to hydrostatic pressure of 40 cm H_2O (bar C) under standard cell culture conditions were similar to levels obtained from control cells maintained on flexible, silastic substrates under 0.3 cm H_2O pressure but no mechanical strain (bar B). Data are mean values ±SEM of three experiments and were normalized with reference (that is, cells maintained on rigid, tissue-culture polystyrene substrates under 0.3 cm H_2O pressure but no mechanical strain; bar A) values taken as 100 percent.

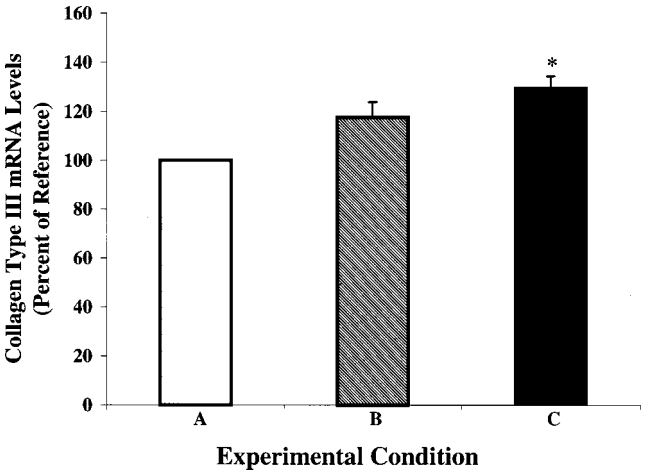

Fig. 5 Normalized collagen Type III mRNA expression by ovine bladder smooth muscle cells simultaneously exposed to mechanical strain of up to 25 percent and to hydrostatic pressure of 40 cm H_2O. Collagen Type III mRNA levels of ovine bladder smooth muscle cells (in serum-free DMEM) on flexible, fibronectin-coated, silastic membranes simultaneously exposed to mechanical strains of up to 25 percent and to hydrostatic pressure of 40 cm H_2O (bar C) under standard cell culture conditions increased by 13 percent compared to levels obtained from control cells maintained on flexible, silastic substrates under 0.3 cm H_2O pressure but no mechanical strain (bar B). Data are mean values ±SEM of three experiments and were normalized with reference (that is, cells maintained on rigid, tissue-culture polystyrene substrates under 0.3 cm H_2O pressure but no mechanical strain; bar A) values taken as 100 percent. *$p<0.1$ compared to control cells maintained on flexible, silastic substrates under 0.3 cm H_2O pressure but no mechanical strain (bar B).

der wall must expose the pertinent cells simultaneously to both hydrostatic pressure and mechanical strain. For this reason, the novel hydrostrain system was designed to simulate specifications pertinent to the bladder and was used in the present study to simultaneously expose bladder smooth muscle cells (cultured on fibronectin-coated, flexible, silastic membranes) to hydrostatic pressure of 40 cm H_2O (a pressure level currently associated with bladder disease and kidney failure) and mechanical strains of up to 25 percent (a level that caused the flexible, silastic membranes to deflect) for 7 h; this system, therefore, established and maintained conditions that better simulated the in-vivo mechanical environment of the bladder wall tissue (specifically, gradual stretching under slowly increasing levels of hydrostatic pressure over a period of 3–4 h).

Literature reports [15] have provided evidence that cells cultured on deflecting membranes experience different magnitudes of strain depending upon their radial location. This was also the case in the present study: radial strain magnitudes (for an applied pressure of 40 cm H_2O) were non-uniform across the silastic membrane surface area; specifically, the average radial strain varied between 5 and 25 percent, with the highest radial strains occurring at the outermost edges of the 10-cm-dia (0.1 cm thick) membrane (Table 1). Variations in radial strain on the silastic substrate, however, provided a reasonable model for the purposes of the present study since bladder tissue is also exposed to various regional mechanical/material conditions in vivo; recent studies used computed tomography (CT) reconstructions and confirmed that extremely complex, and non-uniform, strain fields exist along various regions of the bladder wall tissue [14].

Using the novel in-vitro model, ovine bladder smooth muscle cells were simultaneously exposed to sustained hydrostatic pressure (40 cm H_2O) and mechanical strain (up to 25 percent) for 7 h; this regime of pressure and strain exposure resulted in a 26 percent upregulation ($p<0.01$) of Heparin Binding-Epidermal Growth Factor (HB-EGF) mRNA levels over those obtained from controls (or cells maintained on flexible, fibronectin-coated, silastic membranes under 0.3 cm H_2O pressure but no mechanical strain; Fig. 3). These results are consistent with literature reports of increased HB-EGF levels obtained following exposure of bladder smooth muscle cells to hydrostatic pressure (of 40 cm H_2O) alone [12].

The fact that HB-EGF levels increased with simultaneous exposure to mechanical strain and sustained hydrostatic pressure supports the concept that HB-EGF upregulation within the smooth muscle compartment is an important mechanism by which the bladder wall increases in mass in clinical syndromes involving bladder outlet obstruction. Previous work in our laboratories involving the inhibition of pressure induced bladder smooth muscle proliferation by CRM 197, a specific inhibitor of HB-EGF [17], suggests that this mechanism is primarily autocrine in nature [18].

Compared to levels obtained from control cells (that is, cells maintained on flexible substrates under 0.3 cm H_2O pressure but no mechanical strain), ovine bladder smooth muscle cells exposed simultaneously to mechanical strain and hydrostatic pressure for 7 h expressed similar Collagen Type I mRNA levels (Fig. 4), but increased (by 13 percent) Collagen Type III mRNA levels (Fig. 5). Although Collagen Type I and Type III syntheses were not investigated in the present study, it is reasonable to assume that (aside from post-transcriptional modification of the Collagen RNA signal) increased Type III mRNA levels would result in subsequent increased Collagen Type III protein synthesis and release. These findings are consistent with results from in-vivo animal studies in which long-term outlet obstruction and increased pressures (above 40 cm H_2O) for 6 mo in rabbit bladders resulted in hypertrophy (thickening) of the smooth muscle cell layer, as well as in decreased bladder tissue compliance due to increased (relative to the bladder wet-weight) amounts of connective tissue [19]. The observed decreases in the Collagen Type I:Type III ratio under simultaneous exposure to mechanical strain and hydrostatic pressure in the present study are in agreement with clinically observed changes in the collagen composition of adult human bladders; in those cases, increases in the relative amount of Collagen Type III alone was correlated with decreased bladder tissue compliance [20]. In this respect, increased Collagen Type III mRNA, along with the unchanged mRNA levels of Collagen Type I, observed when bladder smooth muscle cells were exposed simultaneously to pressure and strain in the present in-vitro study, provided the first cellular- and molecular-level evidence of a mechanism behind the decreased bladder wall compliance that accompanies bladder pathologies.

In summary, the present study presented a novel in-vitro system that closely simulates the mechanical environment of the bladder wall tissue that makes possible investigations of the effects of simultaneous exposure of cultured bladder smooth muscle cells to mechanical strain and hydrostatic pressure. The data obtained using the hydrostrain system provided valuable insight into some molecular-level mechanism(s) behind the responses of bladder smooth muscle cells to physiological and to pathological levels of two (that is, hydrostatic pressure and mechanical strain) forces simultaneously. The hydrostrain system and the initial results presented in this paper pertain to conditions with direct relevance to clinical practice. Although more work needs to be completed, these preliminary studies provide evidence that our novel laboratory system is a useful vehicle for future studies aimed at developing novel methodologies for treating bladder and kidney disease.

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References

[1] Maizels, M., 1998, ''Normal and Anomalous Development of the Urinary Tract,'' *Campbell's Urology (Seventh Edition)*, eds., P. C. Walsh, A. B. Retik, E. D. Vaughan, Jr., and A. J. Wein, W. B. Saunders Company, PA, p. 1577.

[2] Matsuno, T., Tokunaka, S., and Koyanagi, T., 1984, ''Muscular Development in the Urinary Tract,'' J. Urol. (Baltimore), **132**, pp. 148–152.

[3] Kaefer, M., Andler, R., Bauer, S. B., Hendren, W. H., Diamond, D. A., and Retik, A. B., 1998, ''Urodynamic Findings in Children With Isolated Epispadias,'' Pediatrics, **102**, 863 (Abstract no. 103).

[4] Lin, V. K., and McConnell, J. D., 1995, ''Molecular Aspects of Bladder Outlet Obstruction,'' *Muscle, Matrix, and Bladder Function*, ed. S. Zedric, Plenum Press, NY, p. 65.

[5] McConnell, J. D., Lee, I. L., and Cher, M. L., 1990, ''Expression of Non-Muscle Caldesmin in Obstruction-Induced Detrusor Hypertrophy (abstr.),'' J. Urol. (Baltimore), **143**, pp. 367–368.

[6] Olivetta, G., Anversa, P., Melissari, M., and Loud, A. V., 1980, ''Morphometry of Medial Hypertrophy in the Rat Thoracic Aorta,'' Laboratory Investigation, **42**, pp. 559–565.

[7] Radzinski, C., McGuire, E. J., Smith, D., Wein, A., Levin, R. M., Miller, F., and Elbadawi, A., 1991, ''Creation of a Feline Model of Obstructive Uropathy,'' J. Urol. (Baltimore), **145**, pp. 859–863.

[8] Wein, A. J., 1992, ''Neuromuscular Dysfunction of the Lower Urinary Tract,'' *Campbell's Urology*, Sixth Edition, eds., P. C. Walsh, A. B. Retik, T. A. Stamy, and E. D. Vaughan, Jr., W. B. Saunders Company, PA, p. 573.

[9] Park, J. M., Borer, J. G., Freeman, M. R., and Peters, C. A., 1998, ''Stretch Activates Heparin-Binding EGF-Like Growth Factor Expression in Bladder Smooth Muscle Cells,'' Am. J. Physiol., **275**, pp. C1247–1254.

[10] Coplen, D. E., Howard, P. S., Duckett, J. W., Snyder, H. M., and Macarak, E., 1995, ''Cultured Bladder Cells and Their Response to Mechanical Strain,'' *Muscle, Matrix, and Bladder Function*, ed. S. Zedric, Plenum Press, NY, p. 207.

[11] Baskin, L. S., Howard, P. S., and Macarak, E., 1993, ''Effect of Physical Forces on Bladder Smooth Muscle and Urothelium,'' J. Urol. (Baltimore), **150**, pp. 601–607.

[12] Kaefer, M., Andler, R., Haberstroh, K. M., Vemulapalli, S., Retik, A. B., Freeman, M. R., and Bizios, R., 1998, ''Physiologic Levels of Hydrostatic Pressure Result in Upregulation of Heparin Binding EGF Like Growth Factor (HB-EGF) in Cultured Neonatal Bladder Smooth Muscle Cells,'' Pediatrics, **102**, p. 863.

[13] Backhaus, B. O., Kaefer, M., Bizios, R., Rink, R. C., Nagatomi, J., and Haberstroh, K. M., 2000, ''Alterations in the Molecular Determinants of Bladder Compliance at Hydrostatic Pressures Below 40 cm H_2O,'' J. Urol. (Baltimore), submitted for publication.

[14] Smith, D. B., Sacks, M. S., Chancellor, M. B., and Block, N. L., 1999, ''*In Vivo* Surface Geometric Analysis of the Urinary Bladder (abstr.),'' *Proc., Bioengineering Conference*, **42 (BED)**, pp. 215–216.

[15] Gilbert, J. A., Weinhold, P. S., Banes, A. J., Link, G. W., and Jones, G. L., 1994, ''Strain Profiles for Circular Cell Culture Plates Containing Flexible Surfaces Employed to Mechanically Deform Cells *In Vitro*,'' J. Biomech., **27**, No. 9, pp. 1169–1177.

[16] Haberstroh, K. M., Kaefer, M., Retik, A. B., Freeman, M., and Bizios, R., 1999, ''The Effects of Sustained Hydrostatic Pressure on Select Bladder Smooth Muscle Cell Functions,'' J. Urol. (Baltimore), **162**, No. 6, pp. 2114–2118.

[17] Mitamura, T., Higashiyama, S., Taniguchi, N., Klagsbrun, M., and Mekada, E., 1995, ''Diptheria Toxin Binds to the Epidermal Growth Factor (EGF)-like Domain of Human Heparin-binding EGF-like Growth Factor/Diptheria Toxin Receptor and Inhibits Specifically Its Mitogenic Activity,'' J. Biol. Chem., **270**, No. 3, pp. 1015–1019.

[18] Haberstroh, K. M., Kaefer, M., and Bizios, R., 2000, ''Inhibition of Pressure Induced Bladder Smooth Muscle Cell Hyperplasia Using CRM197,'' J. Urol. (Baltimore), **164**, No. 4, pp. 1329–1333.

[19] Kato, K., Wein, A. J., Longhurst, P. A., Haugaard, N., and Levin, R. M., 1990, ''The Functional Effects of Long-Term Outlet Obstruction on the Rabbit Urinary Bladder,'' J. Urol. (Baltimore), **143**, pp. 600–606.

[20] Ewalt, D. H., Howard, P. S., Blyth, B., Snyder, H. M., Duckett, J. W., Levin, R. M., and Macarak, E. J., 1992, ''Is Lamina Propria Matrix Responsible for Normal Bladder Compliance?'' J. Urol. (Baltimore), **148**, pp. 544–549.

[21] Griffiths, D. J., van Mastrigt, R., van Duyl, W. A., and Coolsaet, B. L. R. A., 1979, ''Active Mechanical Properties of the Smooth Muscle of the Urinary Bladder,'' Med. Biol. Eng. Comput., **17**, pp. 281–290.