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Shortening velocity is different in longitudinal and circular muscle layers of the rabbit urethra

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Abstract The aim of the study was to investigate whether the functional difference between circular and longitudinal muscles in the female rabbit urethra is reflected in their shortening properties and lactate dehydrogenase (LDH) activity. For mechanical experiments the preparations were chemically skinned to avoid influence of membrane-related mechanisms and to enable maximal activation. Force–velocity relations and the maximal shortening velocity (v_{max}) were determined using the isotonic quick-release method. The v_{max} was three times higher in longitudinal muscle. LDH isoform pattern was determined on agarose gels. The M-subunit, favourable for lactate formation, constituted 70% of the total in both types of muscle. There was no difference in the LDH isoform pattern despite the marked difference in v_{max}. We conclude that the difference in v_{max} reflects differences in the contractile machinery itself. These mechanical characteristics are advantageous for the role of the circular as a tonic muscle contracting during bladder filling, and the longitudinal as a phasic muscle active in opening up the urethra during micturition.

Key words Force–velocity relation · Rabbit · Urethra · Lactate dehydrogenase

Introduction

One function of the urethra is to avoid unintentional leakage of urine. The urethral closure is accomplished by several mechanisms, including passive tone and tonic activation of urethral circular smooth muscle as well as of the striated muscle in the pelvic floor (see e.g. [20]). Most of the time this continence function prevails. During the short periods of micturition the role of the urethra is to facilitate bladder emptying with as little outlet resistance as possible. To accomplish this, neurogenic mechanisms decrease active sphincteric tone. Also, the bladder neck and the proximal urethra are shortened and opened like a funnel. Although the exact mechanism behind this funnelling is not known, it seems to involve activation of longitudinal smooth muscle in the bladder and proximal urethra [5, 10, 11]. There would thus be a difference in contractile activity between the tonically active circular, and the phasically active longitudinally oriented smooth muscle in the bladder outflow region and in the proximal urethra.

Active tone can be produced with a low consumption of ATP if the cross-bridge turnover rate is low. A mechanical correlate to a low turnover rate is a low maximum shortening velocity in the muscle [17]. Smooth muscle has previously been shown to exhibit a wide range of maximal shortening velocity [13]. Also, the maximal shortening velocity can be modulated in one smooth muscle in response to altered functional demands [18]. Mechanical and metabolic properties of the contractile system have been associated with properties of the myosin molecules [6, 13, 18]. Different smooth muscles also exhibit variations in membrane and metabolic properties. "Phasic" muscles are usually fast with a high rate of ATP consumption when activated, whereas "tonic" muscles are generally characterized by slow cross-bridge turnover [19]. There is a correlation between mechanical properties and the isoform composition of lactate dehydrogenase (LDH) enzyme in skeletal muscle [12]. This has also been proposed to exist in smooth muscle [14]; faster smooth muscles having more of the M isoform.

The urethra is interesting in that it has both a circular muscle layer, which is tonically active, and a longitudinal muscle that would be expected to be phasically active. The aim of the present study was to elucidate whether two muscle layers in the same organ can have

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different contractile and metabolic properties. We obtained force–velocity relationships from strips of circular and longitudinal smooth muscle from the rabbit proximal urethra. In order to obtain a lasting and maximal activation of the contractile proteins we used chemically permeabilized muscles where the contractile system can be investigated under controlled conditions. LDH isoforms were determined using agarose electrophoresis.

Material and methods

Animals and preparations

Female rabbits ($n = 6$) weighing approximately 3 kg were killed by cervical fracture. Bladder and urethra were exposed through a midline incision and removed en bloc together with the anterior vaginal wall and transferred to ice-cold physiological salt solution. Strips from both longitudinal and circular muscle layers were prepared from the proximal urethra using an operating microscope (for details see [16]). Preparations from both muscle layers were processed for mechanical experiments and for biochemical analysis as described below.

Determination of LDH isoforms

Samples from both circular and longitudinal muscle layers were weighed and homogenized in a solution containing (in mM): 72 Na_2HPO_4 and 28 NaH_2PO_4 (pH 7.2). The samples were then centrifuged using an Eppendorf (Netheler-Hinz, Hamburg, Germany) 5415 C centrifuge. The supernatant was then used for electrophoretic separation of LDH isozymes essentially as described by van der Helm [21] and Malmqvist et al. [14]. The gels were stained with nitroblue tetrazolium [21] and scanned using a Hoeffer densitometer (Hoeffer Scientific Instruments, San Francisco, Calif.). The relative amount of the LDH isoforms (M_4, M_3H, M_2H_2, MH_3, H_4) as well as relative amounts of M and H isoforms, respectively, was evaluated.

Quick-release experiments

Preparations of circular and longitudinal muscle layers were chemically skinned as described previously [2]. The maximal shortening velocity (v_{max}) as determined using the isotonic quick-release technique (for details see [3, 8]). Briefly, aluminium foil was wrapped around both ends of the muscle fibre preparation. It was then mounted horizontally in a 0.5-ml bath between the arm of a force transducer (AE 801, SensoNor, Horten, Norway, extended with a carbon fibre pin) and an isotonic lever. The experiments were performed at room temperature. The solutions contained (in mM): TES-buffer 30 (pH 6.9), free-Mg^{2+} 2, DTE 1, phosphocreatine (PCr) 12, MgATP 3.2. and 0.5 mg/ml creatine kinase. Ionic strength was adjusted with KCl to 150 mM. Free Ca^{2+} was adjusted by adding EGTA or CaEGTA in appropriate amounts keeping total EGTA at 4 mM. Rigor solutions were made without MgATP and PCr. Length of the preparations was adjusted to give a passive tension $< 5\%$ of maximal active. The muscles were activated by thiophosphorylation of the regulatory light chains as described previously [1]. The preparations were immersed for 15 min in an ATP-free solution containing Ca^{2+} (pCa 4.5), 0.5 μM calmodulin and 2 mM ATP-γ-S. Contraction was initiated by transferring the muscle to Ca^{2+}-free (pCa 9.0) MgATP-containing solution. At the plateau of the contraction a series of releases (12–20) was applied. Two force–velocity relations were determined on each preparation. The muscles were thiophosphorylated between each determination. Force and length signals during each release were digitized at 1 kHz for subsequent analysis using a personal computer equipped with an Analog Devices (Norwood, MA, USA) RTI-800F analogue to digital board. Velocity (v) was determined at a fixed point in time after release (100 ms) as described by Arner and Hellstrand [2]. The afterload (P) and v data were fitted by the Hill equation [9] in the form:

$$v = b(1 - P/P_o)/(P/P_o + a/P_o),$$

as described by Arner [3], where P_o is the isometric force, and a and b are constants. Maximal shortening velocity v_{max}, the intercept with the velocity axis, was calculated as $b\ P_o\ /a$.

Statistics

Statistical comparisons were made using the Student's t-test (two-tailed) for unpaired data. Results are presented as mean ± SEM.

Results

Force–velocity relations

Figure 1, left panel, shows representative force–velocity relations obtained from circular and longitudinal muscle of one urethra. The velocity at all relative afterloads was lower for the circular muscle layer preparation. The maximal shortening velocity (v_{max}), for the whole ma-

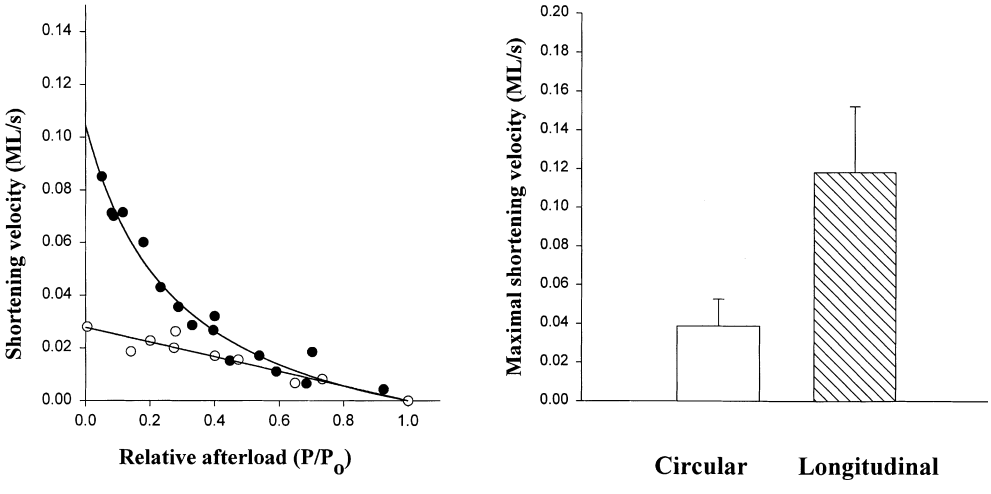

Fig. 1 Force–velocity relationships of circular and longitudinal urethral preparations. The left panel shows data from a circular (*open circles*) and a longitudinal (*filled circles*) preparation. The shortening velocity in muscle lengths per second (ML/s) is plotted against the relative afterload (P/P_o) The Hill force–velocity equation was fitted to the data and extrapolated to obtain the maximal shortening velocity (v_{max}) The right diagram shows mean values for v_{max} in the circular (*open bar*, $n = 5$) and longitudinal (*hatched bar*, $n = 6$) groups. The data were significantly different $P < 0.01$

terial, was about 30% in the circular compared with the longitudinal muscle layer preparations (Fig. 1, right panel).

LDH isoforms

Five different LDH isoforms were demonstrated in both the longitudinal and the circular urethral muscle (Fig. 2). M_3H was the predominant isoform in both muscles. No major differences in the isoform distribution or in the relative amount of the M subunit was observed between the two muscle layers.

Discussion

The smooth muscle in the rabbit urethra is arranged in longitudinal and circular layers. The layers are well defined morphologically and are considered to perform specific actions during bladder filling and emptying. Tonic activation of the circular muscle is one mechanism by which continence is maintained during the long phases of bladder filling (e.g. [20]). In contrast, the longitudinal muscle seems to be involved in the funnelling of the proximal urethra during micturition [5, 10, 11], and is thus active only during the short periods of bladder emptying.

The difference in contractile pattern between the two muscle layers in the urethra may involve differences in innervation, receptor population and/or processes at the level of the contractile machinery. It has been shown that there are clear differences in nerve-mediated functions in the circular and longitudinal muscle layers of the female rabbit urethra [16]. α_1-Adrenoceptors dominated the contractile responses of the circular layer whereas the muscarinic responses were of higher relative importance in the longitudinal muscle.

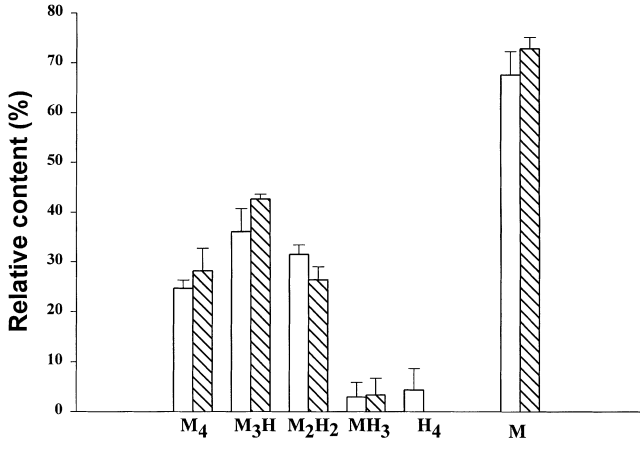

Fig. 2 Lactate dehydrogenase isoform composition in the circular (*open bars*, $n = 5$) and in longitudinal (*hatched bars*, $n = 5$) urethral muscle. The different isoforms (M_4, M_3H, M_2H_2, MH_3 and H_4) are shown in the left, and the relative amount of the M-subunit (M) in the right part. No major differences were detected

Mammalian smooth muscle exhibits a wide range in contractile properties. The maximal shortening velocity (v_{max}) of fully activated chemically skinned muscles, reflects the rate of actin–myosin interaction during filament sliding. v_{max} (At 22°C) varies between approximately 0.02 muscle lengths/s (ML/s) in slow muscles (e.g. rabbit aorta) and about 0.2 ML/s in fast smooth muscles (rabbit rectococcygeus; [13]). A high v_{max} is correlated with a faster rate of tension development in activated muscle [13].

Faster smooth muscles seem to have a lower economy of tension maintenance; i.e. a higher rate of ATP consumption during contraction [17, 7]. Also, faster muscle has a metabolism favouring anaerobic hydrolysis of glucose as reflected by a shift in isoenzymes of LDH to those with a higher relative content of the M-subunit [14].

The aim of the present study was to elucidate whether the different working patterns for the two urethral smooth muscle layers was associated with differences in contraction kinetics and metabolic supply systems. Our results show that the phasically active longitudinal muscle (v_{max} 0.12 ML/s) at 22°C is a comparatively fast smooth muscle whereas the tonically active circular muscle layer (v_{max} 0.04 ML/s) is slow. In vivo the contraction kinetics are influenced by nerve–muscle interaction, intracellular signalling, and properties of the contractile machinery. We have, in our experiments, eliminated the influence of the first two factors since the use of chemically skinned preparations enabled us to achieve a stable and maximal activation of the contractile proteins. The preparations were extended to approximately the same passive tension and we have previously shown that the maximal shortening velocity in skinned smooth muscle is little dependent on preparation length [15]. It is therefore unlikely that the three-fold difference in v_{max} between the muscle layers is due to a difference in preparation extension. Our results thus indicate that the contractile systems in the two layers of the urethra have different kinetic properties. This can reflect differences in cross-bridge turnover rates. Other possibilities such as differences in filament length or orientation cannot be excluded. However, in order to give a three-fold difference in v_{max} the length of the sarcomere equivalents or the contractile filament orientation would have to differ with a factor of 3 or by an angle of 70° ($\cos 70° = 1/3$), respectively. This seems unlikely and we therefore think that the difference in v_{max} reflects a true difference in the actin–myosin interaction. At present we do not know the molecular basis for the difference in cross-bridge kinetics. A different expression of myosin isoforms could be responsible for the difference in v_{max} [13, 18].

For skeletal muscles a correlation exists between the kinetics of the contractile machinery and the LDH isoforms pattern [12]. This relation seems to extend also to the smooth muscle system. For example the rat portal

vein smooth muscle, which is about 10-fold faster than the aorta from the same animal has a 1.4-fold higher relative content of the M isoform [14]. This correlation between v_{max} and LDH isoform pattern is, however, not always observed; e.g. hypertrophy of the urinary bladder smooth muscle results in a slower phenotype with a higher content of the M isoform [14, 18], which suggests that the metabolic system is also influenced by other factors. We found a similar isoform pattern in the two urethral muscles (relative content of M isoform: 70%). The relative content of the M isoform was similar to that found in faster smooth muscles (e.g. about 77% in the rat portal vein; [14]).

In conclusion, the rabbit urethra is a smooth muscle organ which consists of two types of smooth muscle with vastly different maximal shortening velocities. The difference in shortening velocity is not due to activation or structural factors but seems to reflect differences in the contractile machinery itself. From a functional point of view the difference in v_{max} between the two muscle layers is advantageous for their respective roles in continence and micturition.

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