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THE ROLE OF DESMIN IN STRUCTURAL INTEGRITY AND ACTIVE FORCE TRANSMISSION IN HYPERTROPHIC URINARY BLADDER MUSCLE

Aims of Study

Urinary bladder undergoes adaptive growth in a number of pathophysiological conditions associated with mechanical overload. The main initiating factor seems to be stretch of the smooth muscle tissue activating signalling pathways leading to growth. The relative importance of each pathway in the growth response, as well as the mechanical sensor(s) conveying the tissue or cell strain signal to the cell nucleus, are largely unknown.

In smooth muscle the intermediate filaments are coupled to the contractile units through the dense bodies. In the bladder, desmin is the sole component of the intermediate filaments. A striking feature of hypertrophic detrusor smooth muscle cells in both experimental animals and in man is a relative increase in intermediate filaments.

In the present study we have developed a mouse model for bladder hypertrophy in response to partial outlet obstruction, and used this model in desmin deficient mice to answer the following questions: 1. Can adaptive growth of the bladder occur in the absence of intermediate filaments? 2. Are the intermediate filaments required for functional arrangement of the newly formed contractile proteins in the hypertrophic smooth muscle?

Methods

A null mutation in the desmin gene was introduced in the germ line of C57BL/6J. The animals were adult female homozygous (*Des* -/-) transgenic mice and age- and weight-matched wild-type control mice (*Des* +/+). Urinary bladder outlet obstruction was induced by partial ligation of the urethra. Experiments were performed 9-19 days after the partial obstruction. Smooth muscle strips (thickness 0.5 mm, length 4 mm) devoid of urothelium, were cut from the mid-portion of the bladder. The smooth muscle tissue was used either for experiments on intact muscle or chemically skinned. Samples from the smooth muscle tissue were also frozen in liquid N_2 for biochemical analysis or fixed for electron microscopy.

Length-tension experiments: Passive and active (K^+-activation) tension were determined for intact strips at increasing lengths up to lengths well beyond optimum length (l_o) for active force. The strips were then fixed at l_o and embedded in Araldite. The smooth muscle area was measured on sections using a computer-attached video system.

Force-velocity experiments: Skinned preparations were mounted at a length with a just noticeable passive tension, and maximally activated by thiophosphorylation. Isotonic quick releases were performed at various afterloads. Shortening velocity at 100 ms after release was measured. Afterload and velocity were fitted to the Hill equation, and maximal shortening velocity (V_{max}) was calculated.

Quantitative SDS gel electrophoresis: Concentrations of actin and myosin from detrusor extracts were measured on (8 % polyacrylaminde gels) using skeletal actin as standard. Desmin and vimentin in the detrusor muscle were examined using Western blots. Desmin content was expressed relative to actin.

Results

Control Des-/- bladders had slightly lower passive force and significantly lower active force compared to Des+/+. Control Des-/- preparations also had significantly lower V_{max}.

Bladder weight increased about 3-fold after 9-19 days of partial urethral outlet obstruction in both Des-/- and Des+/+ animals. The bladder growth was associated with net synthesis of actin and myosin in both groups. In the hypertrophic Des+/+ bladders the relative content of desmin increased. In Des -/- mice desmin was absent. No alteration in the amount of vimentin was observed. There was no pronounced difference in the contours or cross-sectional area of cells in the corresponding Des+/+ and Des-/- groups. Intermediate filaments were observed in both control and obstructed Des+/+ bladders but were absent in Des-/- bladders. Although Des-/- obstructed bladders were capable of growth following obstruction, 50 per cent of these bladders had partial disruption of the muscle layer with intact mucosa or had hemorrhagic areas of the serosa. Obstruction did not significantly decrease maximum active force in the Des+/+ bladders and did not further decrease the already low maximum active force in the Des-/- bladders. Obstruction decreased V_{max} in the Des+/+ but did not further decrease the already lowered V_{max} in the Des-/- bladders.

Conclusions

1. Although hypertrophic growth of the urinary bladder smooth muscle normally is associated with a marked increase in desmin intermediate filaments, adaptive growth of the detrusor muscle can occur in the absence of such filaments. 2. The intermediate filaments are not required for functional arrangement of the newly formed contractile proteins in the hypertrophic smooth muscle but for active force transmission and maintenance of the wall structure.