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X-BOX BINDING PROTEIN 1 MRNA IS SPLICED IN RESPONSE TO ENDOPLASMIC RETICULUM STRESS IN HUMAN DETRUSOR SMOOTH MUSCLE CELLS

Aims of Study

The urinary bladder requires an adequate supply of oxygen and nutrients via the circulation system in order to function properly. Ischemia of the urinary bladder is reported to be associated with various age-related disorders, such as urinary retention, atherosclerosis, vasospasm, embolization, thrombosis, and with ligation of arteries during pelvic operation. Ischemia, hypoxia and heat shock elicit endoplasmic reticulum (ER) stress and induce unfolded proteins. Accumulation of unfolded proteins in the ER activates a transcriptional induction pathway, termed the unfolded protein response (UPR). The aim of current study was to investigate the UPR by ER stress including hypoxia on cultured human detrusor smooth muscle cells.

Methods

Primary cultures of human detrusor smooth muscle cells were established by an explant method from normal bladder. Immunocytochemistry was used to confirm the identity of the muscle cells by staining specifically for human smooth muscle alpha-actin. The Anaeropack system for cell culture, which was originally designed for the growth of anaerobic bacteria, was used to produce a hypoxic atmosphere for cultured detrusor smooth muscle cells. The oxygen concentration dropped to 1% or less within 1 h. The concentration of carbon dioxide rose to about 5% at 30 min after the induction of the hypoxic conditions, and was maintained at this level for 5 h. No effect of the reaction heat produced by the oxygen absorbent in an airtight jar was recognized. The effects of hypoxia on XBP1 mRNA splicing were examined. We carried out RT-PCR to determine the XBP1 splicing using mRNA prepared from human detrusor smooth muscle cells that had been treated with or without thapsigargin, which evokes ER stress by inhibiting the ER Ca2+ ATPase and tunicamycin, which elicits ER stress by inhibiting protein N-glycosylation, respectively.

Results

5' and 3' primers were set at the positions 412 and 853 of human XBP1 mRNA, respectively, so that the amplified fragment would encompass the overlapping region of next open reading frames. RT-PCR analysis of mRNA untreated cells produced a band of 442bp as expected. In contrast, the same analysis of mRNA from the hypoxia-treated cells produced the band of 442bp plus a band that migrated slightly faster. Both thapsigargin and tunicamycin-treated cells also produced the band of 442bp plus a band that migrated slightly faster. Sequencing analysis revealed that the smaller band lacked internal 26 nucleotides, indicating that XBP1 mRNA is spliced in response to ER stress.

Conclusions

These results suggested that the hypoxia causes ER stress and XBP1 mRNA is spliced in response to hypoxia as the UPR in human detrusor smooth muscle cells.