International Continence Society August 22-26, 1999 29th Annual Meeting Denver, Colorado USA

Category No. 1 | Video Demonstration | Ref. No. 534

Abstract Reproduction Form B-1

Author(s): MP Carey, S de Jong, J Scurry

Double Spacing

Institution City Country: Royal Women's Hospital & Mercy Hospital for Women, Melbourne, Australia

Double Spacing

Title (type in CAPITAL LETTERS): **DETRUSOR INSTABILITY - ARE GAP JUNCTIONS RESPONSIBLE FOR INCREASED MEMBRANE EXCITABILITY?**

Aims of Study

Gap junctions are specialized cell membrane structures, which allow for rapid communication between adjacent cells. They permit the rapid propagation of action potentials between cells. Membrane channels (connexons) of one cell form gap junctions, which are aligned with apposing channels from another cell to form patent water filled passages across two membranes. These connexons are composed of the structural protein connexin. Recent work has identified connexin 43 in the human laboring uterus (1).

Both Brading and Elbadawi have advanced a myogenic basis for detrusor instability. Smooth muscle cells from the detrusor of patients with detrusor instability are more easily excited by direct electrical stimulation when compared with controls. Elbadawi *et al* (2) identified the presence of 'alien' junctions in elderly patients with detrusor overactivity. They concluded that these junctions were de-differentiated gap junctions capable of mediating electrical coupling between detrusor smooth muscle cells and formed the basis of detrusor instability. However, they based their conclusions on electron microscopy findings alone. Cell junctions in more rudimentary forms may be difficult to characterize, increasing the likelihood of misclassification and misinterpretation of function. This problem can be overcome by the additional use of immunohistochemistry to identify the junction types.

The aim of this study was to test the hypothesis that detrusor instability was associated with the presence of gap junctions using an immunoperoxidase technique for the identification of connexin 43. Electron microscopy and vinculin immuniohistochemistry were also used to assist in the identification of detrusor smooth muscle junctions and other membrane structures.

Methods:

Seven women 32 to 68 years old (median 55 years) with severe detrusor instability and no stress incontinence and 5 controls aged 41 to 64 (median 50) with genuine stress incontinence and stable bladders and no symptoms of sensory/urgency or urge incontinence were studied.

Three bladder biopsies, approximately 2-4cm above the trigone and near the midline were taken from each patient. Specimens were numbered and processed for electron microscopy by standard methods. Two investigators blinded to the urodynamic diagnosis analyzed the electron micrographs. Specimens were also processed for immunohistochemistry using an immunoperoxidase technique for the identification of connexin 26 and 43 at an optimal dilution of 1:1,000 and a hydrogen peroxidase labeled antibody to vinculin (dilution of 1:1,750). Infant mouse heart was used as the connexin control and rat small bowel granulation tissue as the vinculin control.

Abstract Reproduction Form B-2

Author(s): MP Carey, S de Jong, J. Scurry

Two investigators assessed the immunohistochemistry independently and were blinded to patient diagnosis during analysis.

Results:

Immunohistochemistry

No cell membrane staining for connexin 26 or 43 was seen in the bladder biopsies of the 7 cases and 5 controls. Entire cell border staining with labeled antibody to vinculin was present in all cases and controls.

Electron Microscopy

No gap junction was identified in any of the cases or controls. Adherens (intermediate) junctions in classic and rudimentary forms were present in all cases and controls. Dense plaques ('hemijunctions') were present on the membranes of all patients and usually a 'complementary' dense plaque could be identified on an adjacent cell membrane. Adherens junctions and dense plaques occupied most of the cell border in all patients. Membrane caevolae occupied the spaces between these adherens junctions and dense plaques.

Conclusion:

Immunohistochemistry and electron microscopy of the detrusor did not identify gap junctions. In organs such as the human uterus and mouse mammary glands, gap junctions have a rapid turnover with formation and involution occurring within several hours (2, 3). Many connexin subtypes have been identified. It could be argued that we failed to identify gap junctions in the overactive bladder because we just happened to biopsy the bladders at a time when the junctions had undergone involution or the junctions were composed of connexin other than 26 and 43.

In this study, the entire cell border stained positive to vinculin confirming that adherens junctions are the predominant and probably only junction present on detrusor smooth muscle cell membranes. Vinculin is a protein associated with adherens junctions and is likely to play an important role in the linkage of actin to the cell membrane. The function of adherens junctions is to mediate mechanical coupling between adjacent cell.

Electron microscopy demonstrated the entire cell border to be occupied by adherens junctions, dense plaques (adherens 'hemijunctions') and caveolae leaving little space for gap junctions. We interpreted 'protrusion junctions' to be rudimentary adherens junctions rather than possible gap junctions as reported by Elbadawi *et al* (2). Connexin 43 was chosen because it has been found in the human uterus and was considered the most likely connexin to be identified if gap junctions were present in the human bladder. Connexin 26 has been identified in human cardiac muscle.

The cause of increased membrane excitability in detrusor muscle in patients with bladder instability remains unanswered. This study failed to demonstrate that detrusor instability is caused by the presence of gap junctions.

References:

1. Acta Obstet Gynaecol Scand 1994: 73: 377-384
2. J Urol. 1994: 150: 1650-1167
3. J Histochem Cytochem. 1994: 42: 931-938