

THE EFFECT OF ISCHEMIA ON BLADDER FUNCTION, AND ACETYLCHOLINE AND ATP RELEASES FROM BLADDER IN WATANABE HERITABLE HYPERLIPIDEMIC RABBITS

Hypothesis / aims of study

Watanabe heritable hyperlipidemic (WHHL) rabbits have atherosclerotic lesions, which are similar to that of human, and have been reported as a good model for evaluation of ischemia-related changes in various tissues. In the present study, we evaluated the effects of ischemia on bladder function and the release of ACh and ATP from bladder strips in WHHL rabbits.

Study design, materials and methods

WHHL rabbits and the age and sex-matched Japanese white rabbits (control group) were prepared. To evaluate bladder function, we measured the volume and the frequency of micturition for three days, and performed the filling cystometry. To examine histological changes, we prepared paraffin blocks of bladder, performed immunohistochemical stainings for S-100 protein and CGRP. Bladder smooth muscle strip was suspended in organ bath filled with Krebs-Henseleit solution, and tension development was recorded. We investigated the effects of carbachol, KCl, and electrical field stimulation (EFS) on bladder strips of both groups. In addition, using the microdialysis technique, microdialysis probe was inserted into the bladder strip, and Ringer solution was perfused into the probe at a constant flow rate of 2.0 μ l/min. Dialysate was collected during EFS (supramaximal voltage, 0.3 msec duration, 40Hz and 3 sec train) and during bladder strip stretch (0 – 40 mN resting tension). The amount of ACh and ATP in the dialysate fraction was measured by HPLC with ECD and luciferine-luciferase assay, respectively.

Results

In WHHL rabbits, the number of micturition was higher and the voided volume was lower than in the control. Cystometrograms of the WHHL rabbits showed premicturition contractions, shorter interval of micturition, lower voided volume, and lower micturition pressure, as compared with the control. In the functional study, the contractions induced by carbachol and EFS in younger WHHL rabbits significantly increased, whereas the responses significantly decreased in older WHHL rabbits. Histological findings showed that urothelium became thinner and connective tissues in muscle layers increased as rabbits grew old. With immunohistochemical stainings, the density of S-100 protein positive neurons significantly decreased and that of CGRP positive neurons significantly increased in the WHHL rabbits. The amount of ACh and ATP released from the bladder strips was lower in the WHHL rabbits than in the control under EFS (neuronal release) and muscle strip stretch (non-neuronal release).

Interpretation of results

The present study demonstrates that ischemia derived from atherosclerosis in WHHL rabbits may cause detrusor overactivity. The cystometric change demonstrates the gradual shift of bladder function from a compensated state to a decompensated state induced by aging and ischemia progression. The decrease in S-100 protein positive neurons may show denervation of motor neurons, and with reduced smooth muscle area it may develop the decrease in micturition pressure. In addition, the increase in CGRP positive neurons implies activation of afferent neurons, and may be related to bladder hypersensitivity. Also, the decreases in neuronal and non-neuronal ACh and ATP releases indicate that significant changes may occur in both efferent and afferent pathways of micturition reflex.

Concluding message

The present study elucidated one of the developmental mechanisms of OAB caused by ischemia and aging. The data suggest that bladder ischemia causes histological changes accompanied by changes in neuronal and non-neuronal ACh and ATP releases, which may influence bladder function and result in detrusor overactivity in WHHL rabbits.

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by The ethics committee of Kumamoto University